Microcirculatory aspects of neurogenic bladder syndrome

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1. INTRODUCTION

The microcirculatory aspects of inflammatory disorders are of importance in the pathology of the urinary system. All the essential elements of inflammation can be traced back to phenomena occurring at the level of the microcirculation. The inflammatory signals at the affected tissues result the alteration of the function of the endothelial cells within the microvessels. In this case, however, activation of the endothelium during the "classic inflammatory response" to antigens is not an initiating factor, but rather a resultant one. In other cases, such as in hypoxia/reoxygenation processes involving oxidative damage, the endothelial layer appears to be the main target of damage. The outcome will be similar in both cases, resulting in endothelial activation and/or endothelial cell dysfunction, characterized by an altered release of endothelium-derived vasoactive mediators and an expression of adhesion molecules with a resultant increase in cell-to-cell interactions, aggravating the microcirculatory processes. In the pathogenesis of endothelial dysfunction, the major role of free radical-mediated reactions can be assumed, such as proteolytic enzymes are likewise of considerable importance. During the process of microcirculatory inflammation, polymorphonuclear leukocytes (PMNs) are also activated, showing an increased expression of adhesion molecules which results in tissue migration of PMNs bringing about additional local tissue damage. Microvascular dysfunction caused by ischemia/reperfusion (IR) injury will eventually (1) manifest in characteristic microcirculatory reactions marked by decreases in (a) functional capillary density, (b) red blood cell velocity, (c) venular and arteriolar diameters, and (d) macromolecular leakage; however, it will also (2) bring about microcirculatory inflammatory reactions that are characterized by PMN-related reactions in the postcapillary venules.

One of the most common forms of bladder inflammation of non-infectious origin is interstitial cystitis (IC), where the tissue damage is initiated in the epithelial layer, causing ulcerous histological changes and a characteristic deep rupture through the mucosa (Hunner's lesion). The barrier lesion is most probably responsible for the clinical signs and symptoms, but hypoperfusion may also play a role in the background of ulcer development in IC. Another common inflammatory disease of the bladder is hemorrhagic cystitis (HC), which is a relatively common iatrogenic side-effect of alkylating chemotherapeutic agents, cyclophosphamide's (CYP) the toxic metabolite (acrolein)

which elicits an inflammatory response of the urothelium affecting the interstitium and microvessels, also with a potential neurogenic component. The tissue infiltration of PMNs and other inflammatory factors has been demonstrated in IC and HC, but the role of microcirculatory reactions in the pathogenesis of IC is unclear.

Neurogenic bladder is a collective term used for all urinary storage and emptying disorders with neurological diseases in the etiology with the most common symptoms being stress incontinence and overactive bladder, involving the bladder and the “bladder outlet". This complex disorder is currently also termed "Neurogenic Lower Urinary Tract Dysfunction". The underlying cause can be diverse ranging from local functional disorders or local nerve injuries, but can also be originating from upper and lower motoneuron injuries or degenerative neurological diseases, which result in disturbances in coordinated functioning of the bladder and the sphincter. It is important to note that “Neurogenic Lower Urinary Tract Dysfunction” manifests in characteristically different symptoms based on the localization of the underlying neurogenic cause. There are two major manifestations of the disease: the overactive (Type #1) and the underactive (Type #2) forms, both of which are accompanied by reduced bladder compliance. The consequences of both of these nervous system processes will ultimately lead to increased bladder pressures, vesico-ureteral reflux, and resultant ureteral and renal dilatations, hydronephrosis, and renal failure. Differential diagnosis is particularly difficult, because overactive bladder can originate not only from neurogenic causes, but can also be idiopathic or myogenic, with unspecific symptoms. Therefore, accurate diagnosis of the severity of urodynamic changes is extremely important to prevent renal damage.

C-fiber chemosensitive primary sensory neurons innervate a variety of organs and tissues and express the transient receptor potential vanilloid type 1 (TRPV1) receptor. The role of TRPV1 receptors is not completely understood yet, but physiological modulatory functions and protective roles have been attributed to these receptors. The activation of the TRPV1 receptor by noxious stimuli results in the release of neuropeptides from the stimulated sensory nerve endings (e.g. calcitonin gene-related peptide; CGRP and substance P) initiating an array of local tissue reactions (vasodilatation and plasma extravasation), i.e. a neurogenic inflammatory response. In the urinary bladder, TRPV1 is expressed richly in the afferent fibers and also in the urothelial cells, and these cells are therefore most probably implicated in sensory signaling and regulation of urinary bladder

functions, including the micturition reflexes. Moreover, several pathological conditions of the urinary bladder, including neurogenic detrusor overactivity, idiopathic detrusor overactivity, and bladder outlet obstruction involve changes in C-fiber afferent mechanisms. In patients suffering from IC, the severity of the clinical symptoms and inflammation correlated with the enhanced expression of TRPV1-immunoreactive nerve fibers in the urinary bladder and urothelial cells from the overactive human bladder exhibit a high capsaicin sensitivity. The involvement of TRPV channels has been demonstrated in cases of overactive bladder and neurogenic detrusor overactivity.

TRPV1 is involved in the transmission of urinary bladder pain and the application of TRPV1 agonists, and by eliminating capsaicin-sensitive sensory nerves, it is of therapeutic use in the treatment of pathological pain conditions of the urinary bladder. TRPV1 activation is known to bring about characteristic microcirculatory reactions in several organs, as the release of vasoactive peptides (calcitonin gene-related peptide, CGRP and substance P), triggering rapid elevation in blood flow and an increase in permeability in different organs. Neurotransmitters released from perivascular nerves are generally implicated in the concept of neurogenic inflammation. The vasodilatory effects are partially linked to the release of CGRP and another, indirect mechanism involves the enhanced activation of endothelial Ca^{2+}-dependent NO synthase and resultant NO release elicited through a TRPV1-dependent rise in intracellular Ca^{2+}. Edema formation elicited by the activation of TRPV1 receptors, however, is linked to the release of substance P and its binding to endothelial neurokinin-1 (NK1) receptors that lead to an increase in vascular permeability and PMN infiltration, where the dose-dependent increase in plasma extravasation could be inhibited by capsazepine or by NK1 receptor antagonism. Even though TRPV1 agonists are used in the therapeutic management of urinary bladder disorders, the potential acute microcirculatory side-effects of TRPV1 activation have not yet been examined. The present study was therefore designed to examine the effects of capsaicin, the archetypal TRPV1 agonist, on PMN–endothelial cell interactions in the microcirculatory system of the rat urinary bladder, using intravital videomicroscopy (IVM) and immunohistochemistry. The contribution of sensory nerves and the sensory neuropeptides substance P and CGRP to these inflammatory phenomena was studied with the aid of selective sensory chemodenervation and pharmacological antagonists.

2. MAIN GOALS OF THE STUDIES

- We aimed to perform a comparative analysis of inflammatory microcirculatory responses of the bladder in animal models under conditions with presumed direct or indirect endothelial damage. More importantly, we compared the microcirculatory consequences of IC and HC with those of bladder IR.
- Even though TRPV1 agonists were used in the therapeutic management of urinary bladder disorders, the potential acute microcirculatory side-effects of TRPV1 activation have not yet been examined. We thus also aimed to investigate the effects of capsaicin, the archetypal TRPV1 agonist, on PMN–endothelial cell interactions in the microcirculatory system of the rat urinary bladder, using IVM and immunohistochemistry. The contribution of sensory nerves and the sensory neuropeptides substance P and CGRP to these inflammatory phenomena was analyzed with the aid of selective sensory chemodenervation and pharmacological antagonists.
- Depending on the cause, neurogenic bladder manifests in both overactive and underactive forms. The accompanying symptoms (i.e. urge incontinence +/- urinary incontinence +/- urinary retention) are not specific to neurogenic bladder, and the clinical cases also show diverse severity. Further, pathophysiological changes in the lower urinary tract could retrogradely damage the ureter and the kidneys. For these reasons, our further aim was to design and propose an algorithm for a rapid and efficient diagnosis and severity assessment of neurogenic bladder in the practice of urology in Hungary. This also aids in choosing a pharmacological or invasive therapeutic approach to protect the upper urinary tract by maintaining low pressure values in the bladder.

3. MATERIALS AND METHODS

The experimental part of the present thesis is based on two series of animal studies. In the first experimental series (Study 1), the microcirculatory consequences of common urological diseases were studied in the rat urinary bladder. In the second experimental study (Study 2), the microcirculatory side-effects and the underlying receptor-dependent

mechanisms of topical capsaicin were examined. In the review article (Study 3), we aimed to set up a diagnostic algorithm to facilitate a proper diagnosis of disease severity and analysis of the urodynamic consequences of neurogenic bladder.

3.1. Study design and experimental protocols (Studies 1-2)

In Study 1, the experiments were conducted in four groups. In the first group, bladder ischemia was performed with reversible ligation of the aa. vesicales for 60 min, and then microcirculatory consequences were examined after an additional 30 min. In the second group, the early phase of IC was induced by 30-minute intravesical instillation of protamine sulfate (PS, 2mg/200µl in physiological saline), which causes selective urothelial damage, under ketamine & xylazine anesthesia by sterile puncture of the bladder dome after laparotomy. Following PS exposure, the bladder was washed with sterile saline and the abdomen was sealed in two layers under sterile conditions. Inflammatory reactions were examined 24 hours after induction. In the third group, HC was modeled, where animals were treated with a high dose of CYP intraperitoneally (40mg kg^{-1}; 0.1ml/100g). Microcirculation studies were performed after 24 hours. Sham-operated animals underwent laparotomy with their bladders instilled with physiological saline.

The aim of the experiments in Study 2 was to examine the microcirculatory inflammatory consequences of a 15-min topical application of capsaicin (50µM) on the bladder surface. We used two major sets. In Set 1, the TRPV1 receptor antagonist capsazepine (CZP, 200µM topically) and ablation of the TRPV1-positive nerves (achieved with the neonatal administration of capsaicin; 50mg kg^{-1} on Day 2 of life) were applied. In Set 2, the selective CGRP receptor antagonist $CGRP_{8-37}$ (10µM) and the NK1 receptor antagonists RP67580 (10µM) were topically applied together with capsaicin to investigate the consequences of selective CGRP and NK1 receptor antagonism. IVM recordings were made 15, 30, and 45 min after topical treatments.

3.2. Intravital microscopy (Studies 1-2)

Microscope: Zeiss Axiotech Vario 100HD microscope; Carl Zeiss GmbH, Jena, Germany; 100-W HBO mercury lamp; Acroplan 20x water immersion objective.

Labeling: fluorescein isothiocyanate (FITC)-labelled erythrocytes (0.2 ml iv), rhodamine 6G-labelled PMNs (0.2%, 0.1 ml iv, Sigma-Aldrich Co, St. Louis, MO, USA)

Analysis: off-line, frame-to-frame, videotaped images (IVM Software; Pictron Ltd, Budapest, Hungary),

Parameters: Red blood cell velocity (μm sec^{-1}), PMN–endothelial cell interactions (rolling, sticking)

3.3. Immunohistochemistry (E-selectin and ICAM-1) (Studies 1-2)

Fixation: 4% paraformaldehyde in a phosphate buffer (0.1M, pH=7.4) for two hours at 4°C, **sectioning:** frozen sections, 15μm in thickness

Labeling: indirect double-labeling

Antibodies: primary: mouse anti-rat anti-ICAM-1 (clone 1A29; BD Pharmingen, San Jose, CA, USA; 1:1000) and goat anti-rat E-selectin (R&D Systems, Minneapolis, MN, USA; 1:1200) dissolved in phosphate-buffered saline containing 0.3% Triton X-100; secondary: DyLight® 488-conjugated donkey anti-mouse and Cy3-conjugated donkey anti-goat secondary antibodies (both from Jackson Immunoresearch Europe, Newmarket, UK). ***mounting:*** ProlongGold medium containing DAPI.

Analysis: 5–9 regions per cryosection, Zeiss LSM 700 confocal microscope ImagePro Plus 6 image analysis software package (Media Cybernetics, USA)

3.4. Methods used to provide a diagnostic algorithm of neurogen bladder (Study 3)

We created an algorithm for the diagnosis of neurogenic bladder syndrome, which includes diagnostic interventions in the order of their proposed sequence, including specific information provided by the actual step (concerning classification and disease severity), based on the current literature data and our own clinical experience.

3.5. Statistical analyses

The minimum number of animals per group was determined based on the "PS: Power and Sample Size Calculator" software package. Data analysis was performed with the SigmaStat statistical software package (Jandel Corporation, San Rafael, CA, USA). In Study 1, differences between groups were assessed with the one-way ANOVA test, followed by the Bonferoni *post hoc test*. In Study 2, changes in microcirculation variables within and between groups were analyzed with the two-way repeated measures ANOVA test, followed by the Holm–Sidak test. Immunohistochemistry data were analyzed using the two-way ANOVA test. All data values are expressed as means ± standard error of the mean (SEM), and P values < 0.05 were considered statistically significant.

4. RESULTS

4.1. Changes in PMN rolling and adhesion in the postcapillary venules of the urinary bladder in different urological models of bladder inflammation (Study 1)

In the first experimental study, direct damage to the bladder endothelium caused by IR resulted in about seven-fold higher values in the number of rolling PMNs as compared to those in the sham-operated group in postcapillary venules. Twenty-four hours after PS instillation, this difference was approximately three-fold; it was approximately five-fold 24 hours after CYP treatment. We observed a significant increase in PMN adhesion in all of the challenged groups, which reached a similar extent (an approximately seven-fold difference when compared to the sham group) in all the groups.

4.2. Changes in red blood cell velocity in the capillaries of the urinary bladder in different urological models of bladder inflammation (Study 1)

Among the different challenges, only IR resulted in significant differences in red blood cell velocity as compared to those after a sham operation. Specifically, IR evoked approximately 30% lower values than those seen in sham animals, whereas red blood cell velocity values were similar to those of the sham group in the IC and HC models (induced by PS and CYP treatments, respectively).

4.3. The effect of selective sensory chemodenervation and TRPV1 antagonism on the capsaicin-induced microcirculatory changes of the urinary bladder (Study 2)

Topical application of capsaicin triggered a marked increase in the degree of PMN–endothelial interactions in the postcapillary venules of the urinary bladder. Both primary (rolling) and secondary (adhesion) interactions were significantly enhanced within 15 min after topical treatment of capsaicin and remained elevated during the entire observation period of 45 min. Life-long sensory chemodenervation by neonatal capsaicin treatment completely prevented the capsaicin-induced vascular changes and indicated that the vascular effects of capsaicin are not direct, but are mediated through the release of vasoactive agents from the sensory nerves. Likewise, the co-administration of capsazepine, a competitive TRPV1 antagonist, markedly inhibited the intravascular inflammatory changes elicited by capsaicin. Lastly, neither capsazepine nor the vehicle for capsaicin produced any changes in PMN-mediated reactions.

4.4. The effects of CGRP and NK1 receptor antagonists on TRPV1 activation-induced microcirculatory reactions of the urinary bladder

In the urinary bladder, the capsaicin-induced increase in leukocyte rolling was prevented with the topical co-administration of the specific CGRP receptor antagonist $CGRP_{8-34}$, but not by the NK1 receptor antagonist RP67580. However, PMN adhesion was significantly reduced by both the CGRP and the NK1 receptor antagonists.

4.5. The effect of capsaicin on the expression of E-selectin and ICAM-1 in the urinary bladder microvasculature

In control urinary bladders, we noticed a faint-to-moderate staining of small venules with antibodies raised against E-selectin and ICAM-1. Topical application of capsaicin on the urinary bladder resulted in a massive increase in both E-selectin and ICAM-1 immunoreactivities in small venules. A quantitative evaluation of the staining intensity revealed a significant rise in the relative pixel intensity for both E-selectin and ICAM-1 immunostaining.

4.6. Recommended diagnostic algorithm for neurogenic bladder syndrome in the sequence of the proposed examination methods

(1) As a first step, an accurate medical history should provide information about the already recognized neurological problem(s) and urinating disorders. (2) Questionnaires and urine diaries aid in an objective assessment of the severity of symptoms. (3) A routine urological physical examination and urine examinations and (4) bladder and kidney ultrasound examinations may detect signs of an advanced stage of the disease, but are not capable of distinguishing between the two types of neurogenic bladder. (5-6) Urodynamic examinations (cystometry and uroflowmetry), however, are indispensable to differentiating between the two major forms of neurogenic bladder, but (7-8) neurological and electromyographic examinations should also be conducted. Using this method, functions of the detrusor muscle and sphincter are examined, involuntary contractions can be detected, and bladder compliance is calculated. (9) Video cystography is appropriate for detecting bladder neck dissynergism, internal sphincter, and bladder neck stenosis.

5. DISCUSSION

5.1. Microcirculatory effects of urological diseases related to direct or indirect endothelial injury

IR injury causes functional injury in the urinary bladder, and microcirculatory changes play a potential role in this process. The causal relationship between functional and microcirculatory changes was confirmed in a study where ischemic preconditioning not only ameliorated the postischemic microcirculatory changes in the bladder, but also prevented deterioration in contractility. In other models of indirect endothelial damage (IC or HC), however, this relationship has not yet been clarified.

The aim of our experiments was to elucidate the reactions at the level of the microcirculation in models where endothelial activation/damage is not a secondary phenomenon. The exact etiology of IC is not fully understood (infection, accumulation of toxic metabolites, urothelial dysfunction, and neurogenic effects). Increased release of pro-inflammatory cytokines and chemokines as well as a beneficial effect of inhibition of their receptors have also been described, suggesting an important role for inflammatory responses in this scenario. In general, inflammatory cytokines modulate both immunological responses and sensory processes that may directly affect the regulation of urination. This line of reasoning is also supported by clinical observations, where increased inflammatory infiltration in IC patients has been reported. Increased chemotactic chemokine receptor signaling can be detected in a HC model, where inhibition of this pathway resulted in reduced bladder hyperexcitability, which is also associated with improved bladder compliance in rats.

When examining microcirculatory inflammatory reactions in models of indirect endothelial injury here, the extent of these changes were compared to those caused by IR injury. It was shown previously in this condition that the disruption of microperfusion and the increase in microvascular permeability are associated with increased cell-to-cell inflammatory responses. PMN-induced damage could be influenced favorably by blocking antibodies used to inhibit PMN adhesion. It is also known that, in addition to urinary urothelium and muscle tissue, vasoconstrictive endothelin receptors located on endothelial cells also play a pronounced role in IR-induced microcirculatory changes in the bladder. In the case of the bladder, reperfusion is characterized by hypoperfusion, which could be derived from (1) the predominance of endothelium-mediated release of

mediators toward vasoconstriction and/or (2) direct endothelial damage as well as (3) an increased edema formation during reperfusion.

The early phase of IC can be well modeled with intravesical instillation of protamine sulfate, which reversibly inactivates the glycosaminoglycan layer. These responses are mediated through epithelial damage and a resultant increase in permeability. In patients with IC, severity of bladder inflammation correlated with not only pain and urgency scores, but also the density of TRPV1-immunoreactive nerve fibers. Leukocyte invasion and mast cell activation have previously been reported in protamine sulfate-induced cystitis, but the related microcirculatory disorder was first described by our group here.

Our present experiments demonstrate that diseases not directly associated with endothelial damage cause microcirculatory disturbances similar to that of IR. The importance of microcirculatory changes in the pathogenesis of IC is also supported by observations where improvement in the bladder barrier function alleviated clinical symptoms with simultaneous reduction in the number of ulcers. There are also assumptions regarding a release of irritating agents, a relative deficiency of NO production and local free radical release in the pathogenesis of the disease. In human studies with IC, significant changes in PMN-derived gene expression and increased tissue accumulation of PMNs as well as of the expression of the adhesion molecule ICAM-1 have been demonstrated.

CYP-induced HC is a very common non-infectious cystitis in clinical practice and characterized by marked bladder edema, hemorrhage, and urothelial damage, whereas the pathophysiological role of cytokines, which are involved in PMN recruitment (TNF-α and IL-1β), is also implicated in the progression of the disease. The presence of the neurogenic component in HC is also thought to be due to increased concentrations of neuropeptides (CGRP and substance P) in the bladder tissue together with an increased expression of TRPV1 mRNA. Bladder barrier dysfunction was shown to be mediated through damage to nerve endings and spinal cord (L6 and S1) cells. The NK1-dependent PMN accumulation was also described in this model which could also be inhibited with TRPV1 antagonism. It should be noted here that these experiments focused on the PMN–endothelial cell interactions in the early phase of the disease, when chemotherapy-related leukopenia cannot develop and the role of PMNs in the later phase of CYP-induced microcirculatory reactions can no longer be assumed.

5.2. Microcirculatory consequences of TRPV1-mediated acute neuropeptide release in the urinary bladder

TRPV1 agonism previously represented one of the possible treatment modalities for overactive bladder syndrome. The cellular inflammatory processes and their dynamics associated with the topical administration of TRPV1 agonists (capsaicin and resiniferatoxin), have been investigated in the bladder here. This study was the first to demonstrate the dynamics of PMN–endothelial interactions following TRPV1 receptor activation evoked by capsaicin in the bladder. An early increase in the frequency of PMN–endothelial interactions was observed in the bladder postcapillary venules as early as 15 min after the administration of capsaicin, and these inflammatory changes persisted throughout the entire experimental period (45 min). The early onset of inflammatory changes following capsaicin administration is consistent with previous observations that found a rapid development of NK1 receptor-dependent edema formation and PMN deposition after capsaicin in the skin, pancreas and trachea.

We have also demonstrated that the microvascular changes were brought about via a specific action of capsaicin on sensory nerves expressing TRPV1 receptors, since capsaicin-induced cellular inflammatory reactions were strongly inhibited by both TRPV1 receptor antagonism and the elimination of TRPV1-expressing nociceptive primary sensory neurons by neonatal capsaicin treatment. These observations suggest a neurogenic origin of the inflammatory process developing in the urinary bladder after the topical administration of capsaicin. The present findings also show that the effects of capsaicin are indirect and brought about by the release of pro-inflammatory neuropeptides known to be released from activated TRPV1-expressing sensory nerves. Both CGRP and substance P contribute to the capsaicin-induced endothelial adhesion of PMNs, but rolling is only initiated by CGRP.

In the urinary bladder, the capsaicin-induced expression of endothelial adhesion molecules manifested in the expression of E-selectin and ICAM-1 immunoreactivities in the postcapillary venules of the urinary bladder occurring in parallel with the cellular microvascular changes. As for the urinary bladder, the NK1 receptor-mediated expression of ICAM-1 occurred as early as an hour after a local arterial injection of substance P. Another potential mechanism of substance P on leukocyte accumulation is via mast cell activation.

Still, little is known about the subcellular regulatory mechanism by which TRPV-related neuropeptide release modulates these changes, and the pathway by which capsaicin achieved these effects remained basically unexplored. The remarkably rapid (less than 60 min) increase in the expression of endothelium-derived adhesion molecules demonstrated here probably cannot be explained by the most time-consuming Ca^{2+}-dependent activation of NF-κB pathways. The present findings may also be of relevance to neurogenic inflammatory processes that develop in other organs under particular pathological conditions. The dynamics and extent of the inflammatory process may depend not only on the sensory innervation density and the distribution of TRPV1 receptors, but also on other factors, such as the density of CGRP and substance P receptors and the adhesion molecule expression of the particular tissue/organ. Lastly, the potential clinical relevance of the present study should be noted, since TRPV1-expressing bladder sensory nerves used to serve as therapeutic targets in the treatment of overactive bladder syndrome and IC in patients that display an overexpression of the TRPV1 receptor in the urinary bladder.

5.3. Suggested sequence of diagnostic interventions aiding assessment of severity and urodynamic features of the two major clinical manifestations of neurogenic bladder. Therapeutic considerations

Differential diagnosis difficulties with neurogenic bladder arise from the fact that many diseases cause symptoms similar to those of neurogenic bladder and findings can be observed in numerous diseases without any neurogenic component. The diagnostic algorithm outlined here can aid in determining the location of the lesion and clarifying whether we are facing an “overactive or flaccid type” of neurogenic bladder, which is the first to differentiate between. Invasive urodynamic examinations are required to detect dynamic, functional changes in urination, supplemented with neurological and electromyographic examinations.

Differential diagnostic considerations

During differential diagnosis, finding the cause of urinary infection is the first step because “simple urinary infection” causes symptoms similar to that of the overactive form of neurogenic bladder. In terms of the hyperactive form of neurogenic bladder, IC, HC, and bladder tumors should be excluded. In IC the symptoms are similar to Type #1 neurogenic bladder with frequent urination, but urethral pain is typical. Furthermore,

cystoscopy confirms Hunner's lesions. HC can be learned from anamnestic data. Urinary bladder tumors also often cause hyperactive bladder-like symptoms, with frequent and urgent urination stimuli and can be differentiated by urine sediment and cytological examinations, bladder ultrasound, and cystoscopy. For Type #2 urinary bladder, prostate hyperplasia and pelvic tumors can also cause similar storage/urination disorders, while urethral stricture and constipation may primarily cause urination problems. In differential diagnosis anamnestic questionnaires, ultrasound scans, and uroflowmetry may help.

Therapeutic considerations

In most cases of neurogenic bladder, symptom relief cannot be achieved by treating the underlying cause; but with approaches used to treat other diseases showing similar symptoms. The main goal of the therapy is to prevent upper urinary tract damage while reducing the patients' subjective symptoms. These test results serve to select the appropriate, optimal and individualized therapy while minimizing potential complications. Among possible therapies of the overactive form of neurogenic bladder, relaxation of the detrusor muscle (antimuscarinic agents), prevention of urinary retention (alpha adrenergic receptor antagonists), improve compliance (tricyclic antidepressant), inhibition of urine excretion (vasopressine) could be an option. Cannabinoids and selective serotonin–noradrenaline reuptake inhibitors may also be considered for the alleviation of symptoms. Modulation of the sensory part of the urination reflex (using capsaicin and resiniferatoxin) is less targeted nowadays because this approach can only succeed when detrusor overactivity is accompanied by an overexpression of TRPV1 in the urothelium and suburothelium. In drug-resistant cases, blockade of the motoric function of the detrusor muscle is feasible with topical application of botulinum toxin. In the case of flaccid bladder, bladder relaxation (via inhibition of alpha adrenergic receptors) can alleviate a weak urine stream and straining. Invasive therapies (e.g. neuromodulatory, sling plastics, urine deviation, artificial sphincters, urethrotomy, and bladder augmentation) can also be considered if pharmacological treatments are ineffective or in the case of serious side-effects. Unfortunately, catheterization/self-catheterization represents the only choice to protect the upper urinary tract in many cases of neurogenic bladder.

6. SUMMARY OF NEW FINDINGS

1. IC and HC are not linked to direct endothelial damage, but characterized by PMN–endothelial interactions within the bladder, similar to microcirculatory inflammatory complications observed in IR, where endothelial damage is a primary pathophysiological event. This observation suggests a potential general role of microcirculatory reactions, uniformly, in the pathogenesis of these diseases.
2. Local capsaicin treatment induces rapid increases in PMN rolling and adhesion and expression of adhesion molecules E-selectin and ICAM-1 in the postcapillary venules of the urinary bladder. These changes can effectively be prevented by sensory chemodenervation with capsaicin and by competitive TRPV1 antagonism. The effect of specific receptor antagonists suggests that TRPV1-induced CGRP release initiates the PMN–endothelial interaction by promoting PMN rolling, but adhesion is mediated by both CGRP and substance P.
3. Based on available literature data and our experience, we propose an algorithm by which diagnosis and differential diagnosis of neurogenic bladder can be conducted in a timely and efficient manner. This may facilitate initiation of optimally tailored and individualized symptomatic treatment of neurogenic bladder with simultaneous minimization of the potential complications in the upper urinary tract. Therefore, this algorithm may be particularly useful for colleagues interested in neuro-urology (neurologists, urologists, and rehabilitation specialists) during their training in Hungary.

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LIST OF FULL PAPERS RELATED TO THE SUBJECT OF THE THESIS

I. **Járomi P**, Szabó A, Garab D, Bodnár D, Uhercsák G, Boros M, Hartmann P. Experimental studies on microcirculatory inflammatory reactions of the urinary bladder. Magy Seb. 2012;65(4):184-90. doi: 10.1556/MaSeb.65.2012.4.3.

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III. **Járomi P**, Banyó T, Boros M, Papp F, Szabó A. Clinical aspects and therapeutic possibilities of neurogenic bladder. Orv Hetil. (IF: 0.53)

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V. **Járomi P**, Hartmann P, Garab D, Bodnár D, Boros M, Szabó A. Inflammatory microcirculatory changes in different experimental models of cystitis in rats. 9th World Congress on Urological Research, 15-17 September 2011, Innsbruck, Austria, WCUR11-0076