Pediatr Pol 2019; 94 (5): 306–310
DOI: https://doi.org/10.5114/polp.2019.89489
Submitted: 6.08.2019; Accepted: 10.09.2019; Published: 31.10.2019

PEDIATRIA Polska
PISMO POLSKIEGO TOWARZYSTWA PEDIATRYCZNEGO
Polish Journal of PAEDIATRICS
OFFICIAL JOURNAL OF POLISH SOCIETY OF PAEDIATRICS

REVIEW PAPER

Urothelium – the brain of the urinary bladder. Will knowing its properties pave the way for creating a tissue-engineered bladder?

Agnieszka Wolny[1], Lidia Hyla-Klekot[1], Agnieszka Pastuszka[2], Grzegorz Kudela[1], Tomasz Koszutski[1]

[1]Department of Paediatric Surgery and Urology, School of Medicine in Katowice, Medical University of Silesia, Katowice, Poland
[2]Department of Descriptive and Topographic Anatomy, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia, Zabrze, Poland

ABSTRACT

The neurogenic bladder is a polyaetiological disease syndrome. It results from changes in the central and peripheral nervous system and, so far, has been mainly regarded at the macroscopic level. An increasing number of recent studies have pointed, however, to the role of intact bladder innervation also at the cellular level. At present, urothelium is considered not only as a tight barrier but also as a complex structure controlling the activity of the urinary bladder due to multiple connections with the nervous, endocrine, and immune systems. As a result of denervation, the biological processes taking place in urothelium are disturbed. Understanding of such signalling pathways could contribute significantly to effective pharmacological treatment of neurogenic bladder dysfunction. Moreover, it may contribute to the creation of a bio-organ that could potentially replace a dysfunctional urinary bladder in such patients.

KEY WORDS:

regenerative medicine, neurogenic bladder, urothelium.

INTRODUCTION

Neurogenic bladder is a polyaetiological disease syndrome with diverse clinical manifestations presenting dysregulation of the detrusor muscle and of the urethral sphincters. The condition is a consequence of changes in both the central and the peripheral nervous systems, and so far it has been discussed mainly at this macroscopic level. However, an increasing number of studies carried out in recent years indicate the importance of intact bladder innervation also at the cellular level. Disorders of the urothelial cell innervation cause significant morphological and physiological changes, which may affect the urinary bladder function.

The urothelium is the part of the mucosal system where constant interaction between the human body and various external factors takes place. Historically, urothelium was considered only as a tight barrier to urine and pathogens from the external environment. Recently the urothelium has been recognised as a more complex organ, both structurally and functionally. It is not only a sealing barrier but has a role as an important communicator between the bladder contents and numerous human homeostasis-regulating systems. The urothelium has the

ADDRESS FOR CORRESPONDENCE:
Agnieszka Wolny, Department of Paediatric Surgery and Urology, School of Medicine in Katowice, Medical University of Silesia, 16 Medyków St., 40-752 Katowice, Poland, ORCID: 0000-0002-4405-0822, e-mail: a.wolny@yahoo.pl

capacity to receive mechanical, chemical, thermal, and biological stimuli from the environment and to transmit them simultaneously to the nervous system [1–5]. Such crosstalk between the urinary and the nervous systems is a highly complex phenomenon. It determines both the normal activity of the bladder muscle and regeneration of the urothelial cells [3]. Understanding the properties of the urothelial cells, including the signalling pathways, will hopefully enable further studies on improvement of neurogenic bladder function.

A great variety of causative diseases makes it very difficult to estimate the actual prevalence of neurogenic bladder. The leading causes of neurogenic bladder are post-traumatic spinal cord injury in adults and myelomeningocele in children. The prevalence of spinal dysraphism has been estimated at 1 per 1000 live births, constituting the second largest group of malformations after heart defects [6, 7]. In 2015, 143,200 children with spinal cord defects were born worldwide [8] while 250,000 to 500,000 people experience the spinal cord traumas each year [9]. The combination of just these two groups results in about 600,000 new patients with neurogenic bladder every year. However, the exact number of such patients seems to be greater.

SENSORY AND TRANSDUCING PROPERTIES OF THE UROTHELIAL CELLS

Although previously underestimated, the urothelium has now been referred to as "the functional centre" of the bladder due to its complex sensory and transducing capabilities [5]. It has been proven that the afferent fibres innervating the bladder (Aδ and C) penetrate into the urothelium and remain in direct contact with the epithelial cells [1, 5, 10, 11]. The axons of the submucosal plexus are very close to the urothelium, while the thinnest of them penetrate throughout its entire thickness [10]. Apodaca *et al.* named such neural network the "uroepithelial-associated sensory web", emphasising that any interruption leads to impaired filling and emptying of the bladder [1]. Chai *et al.* suggested the term "mucosal signalling", pointing to close interaction between the autonomic nerves and the urothelial cells [5]. Tight junctions between the urothelial cells bind them together to form a functional syncytium, which allows for long-distance signal transmission from the urothelial layer to the muscular one, in different directions. This specialised conductive system is responsible for the proper function of the bladder [4, 5].

Expressed on the urothelial cell surface are multiple receptors and ion channels. Some of them play a role in the development of the lower urinary tract, while their expression is determined genetically. Mutations within these genes may lead to diverse malformations of the lower urinary tract [12]. Other cells are analogical to the nociceptors and mechanoreceptors found in other regions of the human body [4]. The urothelial cells receive numerous stimuli, mainly mechanical ones such as intravesical pressure changes, changes in the bladder position, or movements of the neighbouring organs. Stimulation of these receptors triggers activation of transducer proteins, and consequently the signal may be transferred to other cells throughout the bladder wall layers. Multiple transmitters and mediators, modulating activities of sensory neurons, are also released by the urothelial cells [1, 2, 4, 5, 11, 13, 14]. Recognised best is the adenosine triphosphate (ATP), released in response to the bladder wall stretching. Stimulation of its receptors (P2X and P2Y) dispersed in the uroepithelium and in the smooth muscle cells causes various biological effects like sensation of fullness and pain, detrusor muscle contraction, as well as endo- and exocytosis of the umbrella cells [11, 14]. Mechanical stretching of the bladder wall enhances also the release of nitric oxide (NO). Experimental studies have shown that this process depends on the vanilloid receptor (TRPV1), expression of which is common in the urothelium. Its role in induction of the bladder hyperactivity has been proven in infections of the lower urinary tract as well as during normal micturition reflex [14]. Acetylcholine (Ach) is another mediator released in response to mechanical stimuli. It modulates the release of ATP and NO in situ by affecting the urothelial receptors (M2, M3, M5). Ach participates in transduction of the sensory signals, acting upon the afferent sensory fibre receptors (M2, M3), and it triggers bladder muscle contraction by acting directly upon the smooth muscle cells [14]. These receptors are valuable targets for drugs commonly used in pharmacological therapies of the neurogenic bladder [13]. Another molecule attracting scientific interest is nerve growth factor (NGF), produced spontaneously by the urothelium. NGF performs both as a trophic factor and as a signalling molecule [15]. NGF plays a significant role in the generation of pain and urgency in the state of disturbed transduction of sensory information, e.g.: inflammation, hyperactivity, and neurogenicity [11, 15, 16]. The bladder and dorsal ganglions NGF is elevated in spinal cord injury and is correlated with bladder spasticity and its low compliance [13, 15]. These findings will hopefully contribute to the development of a new group of drugs alleviating those inconvenient symptoms.

STRUCTURAL AND FUNCTIONAL CHANGES IN THE UROTHELIUM INDUCED BY DENERVATION

Disrupted communication between the nervous system and the urothelium results in both morphological and functional changes. Spinal cord injury leads to deprivation of the urothelial trophic factors and consequently to abnormal proliferation, differentiation and maturation [17]. Physiologically, the urothelium is characterized by low cellular turnover with the average life span ranging between 6 months and one year [18]. However, it shows

an enormous regenerative potential in response to sudden damage. In case of injury, production of growth factors (especially epidermal growth factor and keratinocyte growth factor) increases, and the damaged cells are more sensitive to these growth factors [3, 16, 18]. Much effort is undertaken to find a progenitor urothelial cell giving rise to all three layers of the uroepithelium and initiating its regeneration. As shown, it is likely to originate from the basal layer and is characterised by the expression of cytokeratin 14 [3, 19]. In denervation, such mechanisms are impaired, resulting in disruption of the urothelium, relaxation of tight junctions between the cells, increased urinary permeability and susceptibility to infection [10, 19, 20]. In the study by Apodaca *et al.* numerous fields of damaged urothelium without any apical umbrella cells were observed as early as two hours after spinal cord injury. After 14 days, initiation of regenerative processes was observed, while on day 28 significant improvement was noted in many experimental animals. Nevertheless, the rebuilt urothelium was not identical and the apical cells were much smaller in size [10]. Recent studies have shown that these small superficial cells do not express cytokeratin 20, a well-known marker of a mature umbrella cell [19]. Similar results were found in studies on expression of cytokeratin 20 in urinary bladder biopsies in patients with spinal cord injuries. Despite the preserved epithelial continuity, cytokeratin 20 was not expressed [21]. This indicated the presence of low-differentiated epithelial areas long after the injury, as a result of chronic mucosal damage due to denervation.

In patients with bladder innervation disorders, regardless of their aetiology, the increased incidence of urinary tract infections was a consequence of the well-known mechanical factors leading to urinary retention and conditions enhancing the excessive growth of bacteria. Increased intra-vesical pressure also has an indirect adverse effect on urothelial cells, leading to their hypoxia and malfunction. Hypoxia-inducible factor (HIF-1α), found in excess in the urothelial cells of patients with neurogenic bladder dysfunction, is a sensitive marker of oxygen deficiency. Together with vascular endothelial growth factor (VEGF), it also triggers further uncontrolled angiogenesis, leading to fibrosis of the bladder and its end-stage disease [22].

Additional factors in the aetiopathogenesis of infections are morphological and functional changes of the urothelium, resulting from its disrupted innervation [23, 24]. The lack of neural stimulation of the urothelium not only disturbs secretion of the growth factors, epithelial regeneration, and function of receptors and adhesion molecules, but also results in malfunction of defence mechanisms, which predispose to infections. Recurrent, chronic, and resistant to treatment, urinary tract infections are among the most severe complications in patients with neurogenic bladder [23, 24]. Experimental studies indicate impairment of immune mechanisms of the bladder wall in rats with spinal cord injury. Over-expression of genes for proinflammatory cytokines and significantly reduced expression of genes regulating the neutrophil recruitment pathways have been observed. These result in increased susceptibility to urinary tract infections, impaired bacterial eradication, and a chronic inflammatory process [20, 25]. Specimens of the bladder wall taken from patients with myelomeningocele show more frequent presence of chronic inflammatory infiltration and decreased expression of uroplakin in umbrella cells, indicating their incomplete differentiation [26]. Another important component of the mucosal anti-inflammatory system is IgA. Immunohistochemical studies of the bladder wall in patients with non-neurogenic dysfunction demonstrated correct representation of IgA in all samples, while biopsies from patients with neurogenic bladder showed such representation in less than half of their number [27]. This confirms the presence of mucosal IgA deficiency in neurogenic bladder.

BLADDER NEUROHISTOLOGY AS THE KEY TO FURTHER DEVELOPMENT IN BLADDER TISSUE ENGINEERING

All recent embryological studies of urothelial cells, their regeneration, and the search for the urothelial progenitor cell have focused on the creation of a bladder by means of tissue engineering. Transplantation of such a bio-organ could replace a fibrotic and non-compliant bladder and hopefully revolutionise the management of neurogenic bladder. However, according to recent studies, this would not be possible without reconstruction of the neural network to ensure correct and constant stimulation of the urothelium.

So far, two methods have been used to create a bladder substitute. The first one used a scaffold of biomaterials, which was expected to become spontaneously settled by the patient's native cells *in vivo* [28]. This would be possible due to the enormous regenerative capacity of the urothelium, able to regenerate even when the entire organ is removed as a result of spontaneous epithelial cell migration from the ureters [29]. The second one assumed implantation of previously prepared scaffolds filled with cells collected from the patient *in vitro* [28]. In 2006, Atala *et al.* reported promising results in seven patients with myelomeningocele, using scaffolds with the urothelial cells of these patients in bladder augmentation. All of the patients showed significantly increased capacity and compliance of the bladder, with no metabolic complications or urinary stones observed and normal production of mucus and the renal function. In the biopsies, normal architecture and cell phenotype were observed [30, 31]. Despite some excellent results, the subsequent 11 paediatric patients with spina bifida, treated with the same method, showed no improvement, and additionally multiple side effects were observed [31, 32]. Given the latest

knowledge, the disappointing results of the study could be explained by the fact that the bladder cells collected from patients and sown on the matrices had been initially damaged due to the lack of proper innervation. It has been repeatedly proven that the urothelial cells collected from the altered bladders present impaired divisions and differentiation *in vitro* [22]. Moreover, no complete regeneration was possible without reconstruction of the urothelial neural network to guarantee the urothelial-mesenchymal crosstalk. A reciprocal interaction between the urothelium, submucosa, and the smooth muscles is crucial in the development of the bladder and begins as early as in foetal life because the urothelium needs signals from the underlying mesenchyme to differentiate properly while the smooth muscle cells can develop only in the company of adjacent properly innervated urothelium [12, 16, 33]. In the period 2010–2014 one research group managed to design and conduct a clinical trial (NCT01087697) evaluating the use of a tissue-engineered artificial bladder conduit. Researchers chose a demanding model of total cystectomy in which both ureters were transplanted to a conduit. It raised hopes in the urology tissue engineering community, but unfortunately the results were not published [34, 35].

The urinary bladder possesses a unique anatomy, allowing for repetitive expansion and contraction. Furthermore, it is lined with a highly specialised multilayer epithelium. The complexity of this structure poses a challenge for regenerative medicine. Beneath the urothelial layer Cajal-like cells act as electrical pacemaker cells that trigger and drive synchronous smooth muscle contraction. Even if we learn how to isolate Cajal-like cells, *in vitro* production of a functional network that could restore the peristaltic wave seems to be far beyond our biotechnological development level [35]. All applied cells are held together by a tri-dimensional scaffold that provides the shape and initial mechanical strength, but further regeneration *in vivo* needs to be induced by molecular signals transported via complicated vascular and nervous networks [36]. The bladder neural plexus fails to regenerate beyond the lesion site and native axons do not elongate into the tissue-engineered graft. In this context, rebuilding the neural network within the neobladder wall should be a high priority [37]. Finding a way to restore proper vascularisation and innervation is the only chance for effective and up-to-date treatment of neurogenic bladder by means of tissue engineering.

CONCLUSIONS

Neurogenicity of the bladder causes structural, functional, and immunological changes in the urothelium. The current results of experimental studies on animal models, as well as investigation of the human bladder sections, indicate that interruption of the innervation of the urinary bladder causes disorders in differentiation, maturation, and function of the urothelium. Interaction between the epithelial cells and the nervous system is a determinant of their continuous complex activity. It also affects the efficiency of the immune system in the bladder wall, including lymphocyte distribution, the amount of mucosal IgA, and the number of cells producing it, which may alleviate the antimicrobial defence. Prevention and treatment of lower urinary tract infections in this group of patients should take into account not only the well-known aetiological factors, such as urinary retention and high intravesical pressure, but also the altered structure and function of the urothelium. Intensive experimental studies of the urothelium morphology and function have some profound practical grounds. The goal of regenerative medicine is to create a urinary bladder that could replace an irreversibly damaged organ. It is known, however, that without restoration of the neural network, correct regeneration of the urothelium and the smooth muscle layer is not possible. Knowledge of the bladder neurohistology is the basis for any further studies in this area.

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Apodaca G, Balestreire E, Birder L. The uroepithelial-associated sensory web. Kidney Int 2007; 72: 1057-1064.
2. Apodaca G. The Uroepithelium: Not Just a Passive Barrier. Traffic 2004; 5: 117-128.
3. Balsara Z, Li X. Sleeping beauty: awekening urothelium from its slumber. Am J Physiol Renal Physiol 2017; 312: F732-F743.
4. Birder L, Andersson K. Urothelial Signaling. Physiol Rev 2013; 93: 653-680.
5. Chai T, Russo A, Yu S, et al. Mucosal signalling in the bladder. Auton Neurosci 2016; 200: 49-56.
6. Botto L, Moore C, Khoury M, et al. Neural-Tube Defects. N Eng J Med 1999; 341: 1509-1519.
7. Pyrgaki C, Niswander L. Neural-Tube Defects. In: Neural Circuit Development and Function in the Brain, Rubenstein J, Rakic P (eds.). Elsevier 2013: 503-519.
8. Blencowe H, Kancherla V, Moorthie S, et al. Estimates of global and regional prevalence of neural tube defects for 2015: a systematic analysis. Ann NY Acad Sci 2018; 1414: 31-46.
9. World Health Organization. Spinal Cord Injury, 2013. http://www.who.int/en/news-room/fact-sheets/detail/spinal-cord-injury (accessed 12.08.2018).
10. Apodaca G, Kiss S, Ruiz W, et al. Disruption of bladder epithelium barrier function after spinal cord injury. Am J Physiol Renal Physiol 2003; 284: F9676-F976.
11. Merril L, Gonzalez E, Girard B, et al. Receptors, channels and signalling in the urothelial sensory system in the bladder. Nat Rev Urol 2016; 4: 193-204.
12. Rasouly HM, Lu W. Lower urinary tract development and disease. WIREs Syst Biol Med 2013; 5: 307-342.
13. Cruz C, Cruz F. Spinal Cord Injury and Bladder Dysfunction: New Ideas About an Old Problem. Sci World J 2011; 11: 214-234.
14. Winder M, Tobin G, Zupancic D, et al. Signalling molecules in the urothelium. BioMed Res Int 2014; 2014: 297295.

15. Korzeniecka-Kozerska A, Porowski T, Michaluk-Skutnik J, et al. Urinary nerve growth factor level in children with neurogenic bladder due to myelomeningocele. Scand J Urol 2013; 47: 411-417.
16. Baskin L, Hayward S, DiSandro M, et al. Epithelial-mesenchymal interactions in the bladder. In: Advances in bladder research, Baskin L, Hayward S (eds.). Springer Science & Business Media 1999: 49-62.
17. Vaidyanathan S, McDicken I, Soni B, et al. Possible role of denervation-induced changes in the urothelium in the pathophysiology of cystitis in patients with spinal cord injury: a hypothesis. Spinal Cord 1997; 35: 708.
18. Staack A, Hayward S, Baskin L, et al. Molecular, cellular and developmental biology of urothelium as a basis of bladder regeneration. Differentiation 2005; 73: 121-133.
19. Kullmann F, Clayton D, Ruiz W, et al. Urothelial proliferation and regeneration after spinal cord injury. Am J Physiol Renal Physiol 2017; 313: F85-F102.
20. Balsara Z, Ross S, Dolber P, et al. Enhanced susceptibility to urinary tract infection in the spinal cord-injured host with neurogenic bladder. Infect Immun 2013; 81: 3018-3026.
21. Vaidyanathan S, McDicken I, Ikin A, et al. A study of cytokeratin 20 immunostaining in the urothelium of neuropathic bladder of patients with spinal cord injury. BMC Urology 2002; 2: 7.
22. Radford A, Hinley J, Pilborough A, et al. Hypoxic changes to the urothelium as a bystander of end-stage bladder disease. J Pediatr Urol 2019; 15: 158.e1-158.
23. Humberto R, Hickling D. Urinary tract infection in the neurogenic bladder. Transl Androl Urol 2016; 5: 72-87.
24. Vasudeva P, Mandersbacher H. Factors implicated in pathogenesis of urinary tract infections in neurogenic bladders: some revered, few forgotten, others ignored. Neurourol Urodyn 2014; 33: 95-100.
25. Chaudhry R, Madden-Fuentes R, Ortiz T, et al. Inflammatory response to Escherichia coli urinary tract infection in the neurogenic bladder of the spinal cord injured host. J Urol 2014; 191: 1454-1461.
26. Schlager TA, Grady R, Mills S, et al. Bladder epithelium is abnormal in patients with neurogenic bladder due to myelomeningocele. Spinal Cord 2004; 42: 163-168.
27. Vaidyanathan S, McDicken I, Soni B, et al. Secretory immunoglobulin A in the vesical urothelium of patients with neuropathic bladder – an immunohistochemical study. Spinal Cord 2000; 38: 378-381.
28. Adamowicz J, Pokrywczynska M, Van Breda S, et al. Concise Review: Tissue Engineering of Urinary Bladder; We Still Have a Long Way to Go? System Cells Transl Med 2017; 6: 2033-2043.
29. Pokrywczynska M, Jundzill A, Adamowicz J, et al. Inżynieria tkankowa – eksperymentalna metoda regeneracji pęcherza moczowego. Postepy Hig Med Dosw. 2013; 67: 790-799.
30. Atala A, Bauer S, Soker S, et al. Tissue-engineered autologous bladders for patients needing. Lancet 2006; 367: 1241-1246.
31. Colombo F, Sampogna G, Cocozza G, et al. Regenerative medicine: clinical applications and future perspectives. J Microsc Ultrastruct 2017; 5: 1-8.
32. Joseph D, Borer J, De Filippo R, et al. Autologous cell seeded biodegradable scaffold for augmentation cystoplasty: phase II study in children and adolescents with spina bifida. J Urol 2014; 191: 1389-1395.
33. Southgate J, Harnden P, Selby P, et al. Urothelial tissue regulation. Unraveling the Role of the Stroma. In: Advances in bladder research, Baskin L, Hayward S (eds.). Springer Science & Business Media 1999: 19-29.
34. Clinical Trial NCT01087697. https://clinicaltrials.gov/ct2/show/NCT01087697 (accessed 1.09.2019).
35. Adamowicz J, Van Breda SV, Kloskowski T, et al. Constructing artificial urinary conduits: current capabilities and future potential. Expert Rev Med Devices 2019; 16: 135-144.
36. Horst M, Eberli D, Gobet R, et al. Tissue engineering in pediatric bladder reconstruction – the road to success. Front Pediatr 2019; 7: 91.
37. Adamowicz J, Kuffel B, Van Breda SV, et al. Reconstructive urology and tissue engineering: Converging developmental paths. J Tissue Eng Regen Med 2019; 13: 522-533.