available at http://ajws.elsevier.com/tcmj

Tzu Chi Medical Journal

ELSEVIER

Review Article

Pathophysiology of Urothelial Dysfunction in Patients with Interstitial Cystitis/Painful Bladder Pain Syndrome: Increased Apoptosis and Decreased Junctional Protein Expression in the Urothelium due to Suburothelial Inflammation

*Jia-Heng Shie, Hann-Chorng Kuo**

Department of Urology, Buddhist Tzu Chi General Hospital and Tzu Chi University, Hualien, Taiwan

Article info

Article history:
Received: January 23, 2009
Revised: February 2, 2009
Accepted: February 3, 2009

Keywords:
Apoptosis
Inflammation
Interstitial cystitis
Painful bladder syndrome
Urothelial dysfunction

Abstract

Interstitial cystitis/painful bladder syndrome (IC/PBS) is a heterogeneous syndrome characterized by bladder pain and associated with frequency and nocturia. Recent findings have proposed several pathophysiological mechanisms including epithelial dysfunction, activation of mast cells, neurogenic inflammation, autoimmunity and occult infection. The human bladder urothelium and urothelial cells play an important role in the normal defense mechanism. One of the most common findings in IC/PBS patients is denudation or thinning of the bladder epithelium, suggesting an altered regulation of urothelial homeostasis. Urine from patients with IC/PBS has been shown to inhibit urothelial proliferation through antiproliferative factor (APF). In urine samples of IC/PBS patients, significant increases in APF, decreases in heparin-binding epidermal growth factor (HB-EGF) and increased levels of EGF were discovered. APF expressed by the urothelial cells induces increased permeability in cell culture, and regulates expression of other cytokines, including upregulation of HB-EGF and downregulation of EGF. These cytokine abnormalities were also related to increases in purinergic signaling, which mediates increased bladder sensation. Abnormal expression of uroplakin, chondroitin sulfate and tight junctional protein zonula occludens-1 (ZO-1) strongly suggests abnormal differentiation in bladders with IC/PBS, whereas elevated E-cadherin expression may represent an adaptation to increased bladder permeability. The epithelial damage may precede the other histopathologic findings in the bladder wall. A local inflammatory process might be induced through the afferent and efferent nerves in the suburothelial interstitial cellular network which integrate the transmission of signals from the urothelium to the detrusor muscles in the bladder wall. Investigation of the relationship between chronic inflammation and urothelial dysfunction such as urothelial apoptosis, expression of junctional protein and inflammatory reactions in the suburothelium might demonstrate this hypothesis. (*Tzu Chi Med J* 2009;21(2):103–109)

*Corresponding author. Department of Urology, Buddhist Tzu Chi General Hospital, 707, Section 3, Chung-Yang Road, Hualien, Taiwan.
E-mail address: hck@tzuchi.com.tw

1. Introduction

Interstitial cystitis/painful bladder syndrome (IC/PBS) is a chronic bladder condition seen mainly in women (10:1 female to male). It is characterized by bladder pain, frequency and nocturia. There is no clear definition of IC/PBS as the clinical symptoms and histology are not specific. The diagnosis of IC/PBS can only be based on a combination of a thorough patient history, sterile and cytologically negative urine, cystoscopic hydrodistention under anesthesia and bladder biopsies. Many different etiologies have been proposed, including: (1) a post-infection autoimmune process; (2) mast cell activation induced by inflammation, toxins or stress; (3) urothelial dysfunction and increased permeability of the urothelium; and (4) neurogenic inflammation. However, none of these etiologies has been definitely proven, and therefore, no single treatment has been reported to have long-term effects in eradicating symptoms of this mysterious bladder disorder [1].

2. Chronic inflammation in IC/PBS

The cause of IC/PBS has been considered to result from long-standing inflammation of the bladder. Bladder histological analysis shows infiltrates of mast cells, eosinophilic leukocytes and T-lymphocytes. This suggests that the disease is mediated by the immune system [2]. However, the triggering factor that leads to disease is still unknown. In previous reports, the histological evidence has shown several marked changes in various tissue elements. First, abnormal behavior of urothelial cells disrupts the permeability barrier. Second, vascular lesions include endothelial cell injury and suggest a slow microcirculation. Third, neural changes include a combination of degenerative and regenerative features [3,4].

Previous reports indicate that the urothelium plays a pivotal role as a barrier between urine and its solutes and the underlying bladder. Bladder surface mucus is a critical component of this function [5–7]. The biologic activity of mucus that imparts this barrier function is generated by the highly anionic polysaccharide components (glycosaminoglycans), which are extremely hydrophilic and trap water at the outer layer of the umbrella cell. In patients with IC/PBS, disruption of the urothelial barrier may initiate a cascade of events in the bladder, leading to symptoms and disease. Specifically, urothelial dysfunction leads to the migration of urinary solutes, in particular, potassium, which depolarizes nerves and muscles and causes tissue injury [8,9]. Consequently, it is imperative to understand the biologic effects by which the urothelium changes its growth behavior and the expression pattern of signal transduction molecules under these effects.

The chronic pain symptomatology in IC/PBS may also be due to central nervous system sensitization and persisting abnormality or activation of the afferent sensory system in the urinary bladder [10]. Increased central c-fos expression has been demonstrated in animal models of neurogenic detrusor overactivity and chronic inflammation. Elimination of rat spinal neurons expressing neurokinin 1 receptors reduces bladder overactivity and spinal c-fos expression induced by bladder irritation [11,12]. If the neurogenic inflammation in the dorsal root ganglia can be eliminated gradually through intravesical treatment, the visceral pain in IC/PBS can thus be relieved.

Recent findings have proposed several pathophysiological mechanisms, including epithelial dysfunction, activation of mast cells, neurogenic inflammation, autoimmunity and occult infection. Therefore, treatments targeting these pathophysiologies have been investigated [13]. One of the most common findings in bladder mucosal biopsies from IC/PBS patients is denudation or thinning of the bladder epithelium, suggesting an altered regulation of urothelial homeostasis. Other bladder abnormalities include increased nerve fiber density and inflammatory cell infiltration. Previous reports on bladder biopsies of patients with IC/PBS have confirmed the involvement and presence of eosinophils, macrophages in the urothelium and mast cells in the detrusor. Involvement of eosinophils is also supported by urine cytology showing increased urinary eosinophil cationic protein in the urine of patients with IC/PBS [14,15]. Mast cells have been considered as crucial effector cells for the immune response implicated in the pathogenesis of IC/PBS [2].

3. Biomarkers for IC/PBS in the bladder and urine

IC/PBS was found to be associated with increased urinary adenosine triphosphate (ATP) and increased stretch-activated ATP release by bladder urothelial cells, suggesting augmented purinergic signaling in the bladder, which could be blocked by suramin and heparin-binding epidermal growth factor-like growth hormone (HB-EGF) [16]. Single-cell electrophysiological studies revealed that the strong inward rectifying potassium current with conductance of the Kir2.1 channel was significantly reduced in IC/PBS bladder urothelial cells. Epidermal growth factor (EGF) caused a dose-dependent decrease in the inward potassium current. Treatment of IC-bladder urothelial cells (BUC) with HB-EGF significantly increased the current. Changes in BUC membrane potassium conductance caused by altered levels of EGF and HB-EGF may play a role in the pathophysiology of IC/PBS [17].

Antiproliferative factor (APF) is a small glycoprotein made specifically by bladder epithelial cells in patients

with IC/PBS that induces changes in expression of certain epithelial cell proteins and profoundly inhibits cell growth. APF may affect the increased permeability and decreased tight junction formation of bladder epithelial cells and contribute to the urothelial leak and bladder pain symptoms seen in IC/PBS [18]. In urine samples of patients with IC/PBS, significant increases in APF, decreases in HB-EGF and increased levels of EGF were discovered [19]. Urine from patients with IC/PBS has been shown to inhibit urothelial proliferation through APF and to contain decreased levels of HB-EGF compared to controls. Hydrodistention of bladders with IC/PBS significantly increased urinary HB-EGF toward control values and decreased urinary APF activity, but APF levels at 2 weeks after hydrodistention were still significantly higher than in controls [20].

Several previous studies reported the discovery of APF which is made uniquely by bladder epithelial cells from patients with IC/PBS and profoundly inhibits normal bladder epithelial cell growth [21]. Microarray analysis indicated that APF can also induce changes in the pattern of cellular gene expression toward a more differentiated phenotype. Identification of this factor is therefore important for determining its potential role in the pathogenesis of IC/PBS and establishing its utility as a biomarker for this disease [19]. Previous results indicate that APF treatment causes significant increases in the paracellular permeability of normal bladder epithelial cell monolayers and the attenuation of tight junctions compared to mock APF, similar to changes seen in IC/PBS cells. Because of its apparent effects on bladder epithelial cell tight junctions and paracellular permeability *in vitro*, APF may contribute to the leakiness of the bladder epithelial barrier seen in IC/PBS [22].

The human bladder urothelium and urothelial cells play an important role in the normal defense mechanism. APF expressed by the urothelial cells induces increased permeability in cell culture, and regulates expression of other cytokines, including upregulation of HB-EGF and downregulation of EGF. These cytokine abnormalities are also related to increases in purinergic signaling, which mediates increased bladder sensation. Alterations of uroplakins, a glycoprotein expressed only in the apical urothelial cells, may result in bladder symptoms related to increased permeability or decreased protective function [23].

4. Urothelial dysfunction in IC/PBS

Studies of urothelial differentiation in IC/PBS also demonstrated that the acquisition of a transitional cell morphology occurred in some IC-derived cell lines, suggesting that a subset of patients with IC/PBS might have a failure of urothelial cytodifferentiation, which might contribute to the disease and bladder dysfunction [5]. Abnormal expression of molecular markers has been found in IC/PBS bladder biopsies. Abnormal expression of uroplakin, chondroitin sulfate and tight junctional protein zonula occludens-1 (ZO-1) strongly suggests abnormal differentiation in bladders with IC/PBS, whereas elevated E-cadherin expression may represent an adaptation to increased bladder permeability [24].

Ki-67 is a commercially available monoclonal antibody that reacts with a nuclear antigen expressed in proliferating cells but not in quiescent cells. Expression of this antigen occurs preferentially during the late G1, S, G2, and M phases of the cell cycle, while in cells in the G0 phase the antigen cannot be detected [25]. To confirm the results of a TUNEL stain, we compared the ratio of proliferating and apoptotic cells in a pathologic assay (Fig. 1). The preliminary data showed that Ki-67 positive cells are rare in IC/PBS patients compared with controls (Fig. 2). These results are consistent with previous reports.

Vascular endothelial growth factor (VEGF), which plays a key role in bladder inflammation, is closely associated with the vascular alterations observed in patients with IC/PBS. In addition, recent findings indicate that VEGF-Rs and co-receptors (neuropilins; NRP) are strongly expressed in both the human bladder urothelium and in a human bladder cancer cell line (J82) and that the expression of NRP2 and VEGF-R1 is significantly downregulated in subjects with IC/PBS compared with control subjects [4]. These results suggest that urinary VEGF may gain access to the bladder wall via their receptors. According to this evidence and the inflammation data from histological analysis, investigating the relationship of VEGF proteins and their receptor/co-receptor in the abnormal urothelium of patients with IC/PBS might clarify the role of VEGF in the pathogenesis of urothelial dysfunction in IC/PBS.

In previous research, some evidence has strongly suggested that abnormal differentiation in the IC/PBS urothelium with a loss of E-cadherin and altered differentiation markers is independent and occurs independently of inflammation [26]. But some results show a less proliferative phenotype, with increased expression of E-cadherin in IC/PBS. Some evidence showed that elevated E-cadherin may represent an adaptation to increased bladder permeability [18,24]. Our preliminary results indicated that the distribution of E-cadherin is different in patients with IC/PBS with different maximal bladder capacities (Fig. 3). Therefore, the role of E-cadherin in the pathophysiology of IC/PBS is still controversial. We need more molecular and histological evidence to investigate the role of E-cadherin in IC/PBS.

Previously published results and data confirm that IC/PBS involves an aberrant differentiation program in the bladder urothelium that leads to altered synthesis

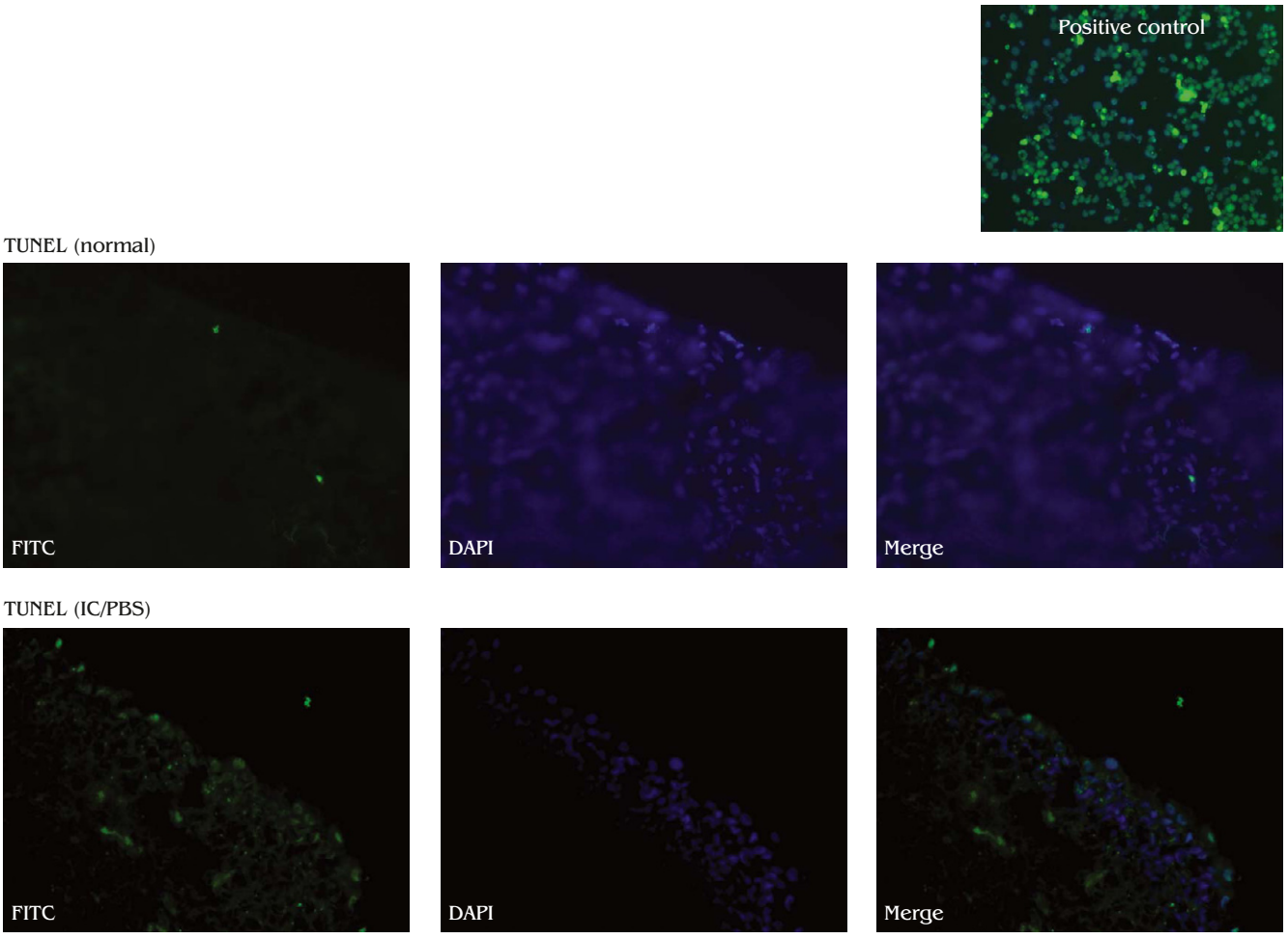

Fig. 1 — TUNEL stain shows that the number of apoptotic cells is slightly increased in patients with IC/PBS compared with normal controls.

of several proteoglycans, cell adhesion and tight junction proteins, and bacterial defense molecules such as GP51. Therefore, replacement therapy with glycosaminoglycans has been widely used for treatment of IC/PBS [27]. However, further correlation of the expression of the proteoglycan core proteins and differentiation related markers with inflammation scores in IC/PBS bladder biopsies revealed that the abnormalities in urothelial differentiation and loss of barrier function in IC/PBS were independent of inflammation [26].

Expression of intercellular adhesion molecules ICAM-1, P-selectin, and E-selectin is highly positive in bladders with IC/PBS but not in controls. ICAM-1 binds to cellular adhesion molecules (CAMs), which is necessary for initiation of inflammation. The results support different degrees of bladder inflammation in IC/PBS [28]. Increased ICAM-1 intensity was found in IC/PBS patients. The ICAM-1 intensity was higher in those who responded to bladder hydrodistention plus hyaluronic acid instillation. By blocking the ICAM-1 receptors, hyaluronic acid presumably alleviates the inflammatory process [29].

Bladders with IC/PBS showed morphological changes indicative of apoptosis. TUNEL staining showed apoptotic cells in the microvascular endothelial cells but not in the endothelial cells of venules. Bladder microvascular endothelial cells may play an important role in the pathogenesis of IC/PBS [30]. Bladder epithelial cells from patients with IC/PBS exhibited profoundly decreased proliferation, decreased expression of cyclic D1 and JNK and increased paracellular permeability compared to normal urothelial cells. Experiments in serum-free medium showed that the proliferation rate of explanted bladder epithelial cells from patients with IC/PBS was significantly decreased compared to that of control cells, indicating an intrinsic abnormality in IC/PBS cell proliferation. This abnormality may be caused by APF, which induces reversible inhibition of HB-EGF-like growth factor production and normal bladder epithelial cell proliferation [31]. APF treatment caused significant increases in the paracellular permeability of normal bladder epithelial cell monolayers and the attenuation of tight junctions compared to mock APF, similar to changes seen in IC cells. APF treatment

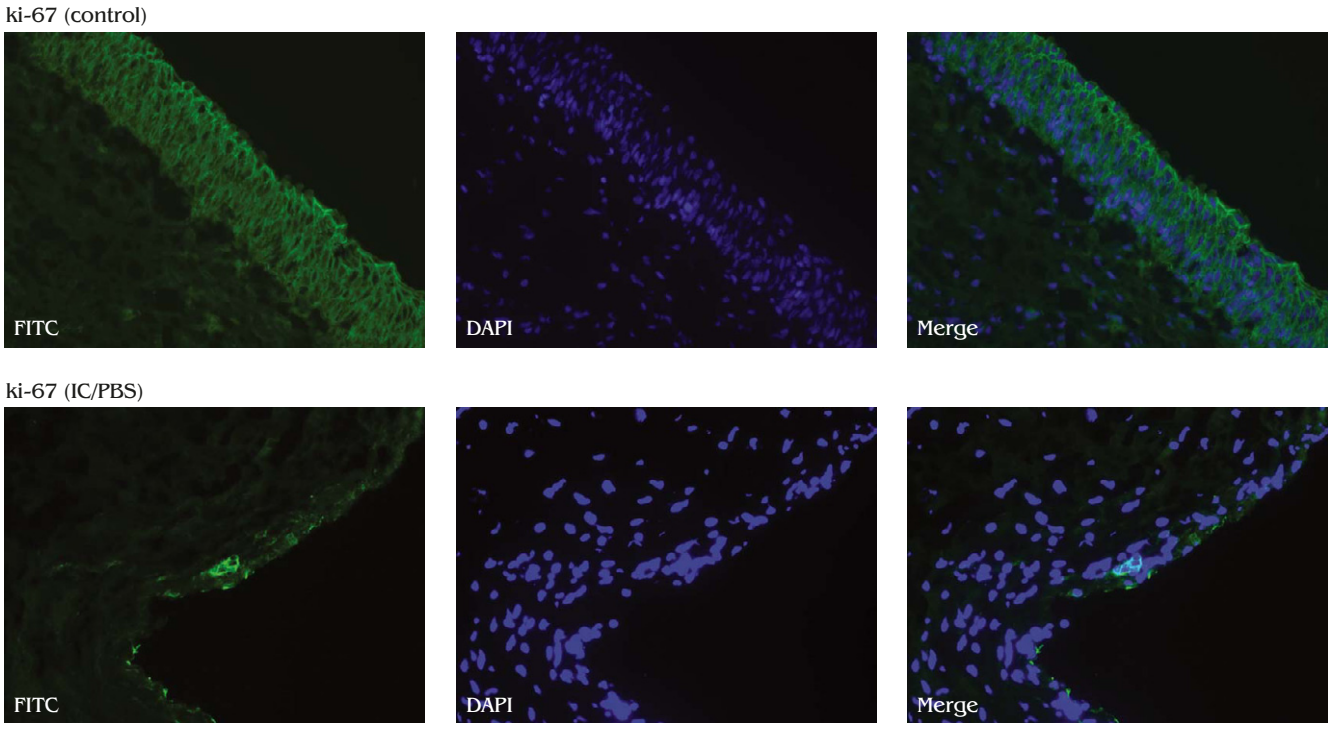

Fig. 2 — The signal of Ki-67 in IC/PBS is dramatically reduced compared with that in normal controls. This result indicates that the differentiation and growth rate of urothelium in IC/PBS is abnormally decreased.

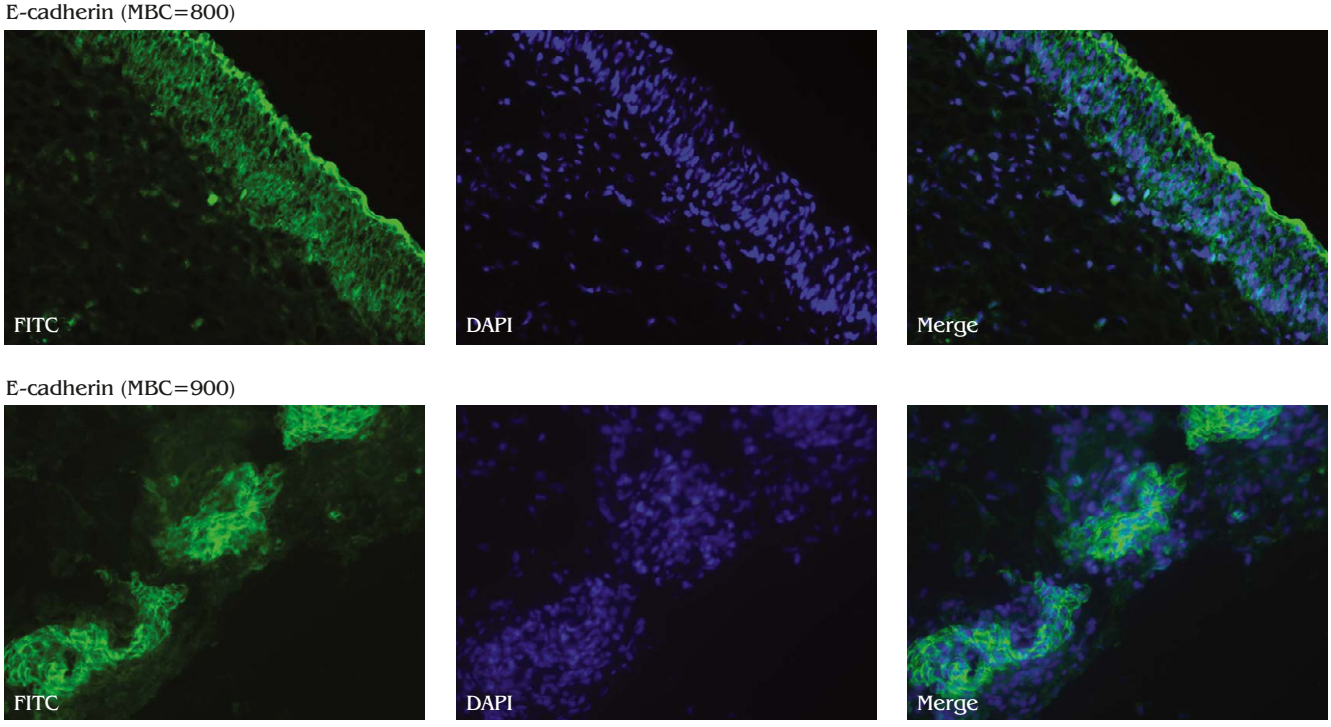

Fig. 3 — Immunohistochemistry study of E-cadherin shows that the distribution of E-cadherin is different in patients with IC/PBS with different bladder capacities.

also decreased expression of the tight junction proteins zonula occludens-1 and occludin [18,32].

5. Effect of chronic inflammation on urothelial dysfunction

Chronic suburothelial inflammation might inhibit normal basal cell proliferation and affect apical urothelial function. Treatment of urothelial dysfunction cannot be based solely on replacement of defense glycoproteins in the bladder urothelium. Furthermore, bladder inflammation caused by intravesical irritants or in patients with IC/PBS leads to acute afferent nerve activity and to long-term plasticity that lowers the threshold for nociceptive and mechanoceptive afferent fibers [33,34]. Chronic sensitization of afferent fibers might involve both peripheral and central mechanisms. A rise in bladder nerve growth factor in the muscle or urothelium initiates signals that are transported along the afferent nerves of the bladder to the dorsal root ganglion or spinal cord [35,36]. Based on these data, successful treatment of IC/PBS should aim at several targets including urothelial defense defects and suburothelial inflammation.

Previous studies showed that intravesical injection of botulinum toxin A (BoNT-A) reduced bladder pain in patients with refractory IC/PBS [37,38]. Nerve growth factor levels in the bladder tissue are significantly increased in patients with IC/PBS and decreased to the normal range after BoNT-A treatment [39]. Repeated BoNT-A injections plus hydrodistention might provide a better outcome in treating IC/PBS. If repeated BoNT-A injections can relieve bladder pain and increase bladder capacity in responders, the result might provide evidence of urothelial repair and reduction of suburothelial inflammation in IC/PBS responders. Chronic suburothelial inflammation might alter urothelial function and cell differentiation, and BoNT-A injection might reduce the inflammation and restore a healthy urothelium, thereby improving the clinical symptoms of IC/PBS.

6. Conclusion

Investigation of the relationship between chronic inflammation and urothelial dysfunction such as urothelial apoptosis, expression of junctional protein and inflammatory reactions in the suburothelium of patients with IC/PBS might demonstrate the hypothesis that suburothelial inflammation affects urothelial function. Treatment of IC/PBS by surface protective agents without an anti-inflammatory effect might not adequately eradicate the pathophysiology of IC/PBS. Investigating changes in urothelial dysfunction and suburothelial inflammation at baseline and after BoNT-A injections can provide evidence for the existing pathophysiology of IC/PBS.

References

1. Hanno PM, Sant GR. Clinical highlights of the National Institute of Diabetes and Digestive and Kidney Diseases/ Interstitial Cystitis Association scientific conference on interstitial cystitis. *Urology* 2001;57(Suppl 6A):2–6.
2. Sant GR, Kempuraj D, Marchand JE, Theoharides TC. The mast cell in interstitial cystitis: role in pathophysiology and pathogenesis. *Urology* 2007;69(Suppl 4):34–40.
3. Van De Merwe JP, Arendsen HJ. Interstitial cystitis: a review of immunological aspects of the aetiology and pathogenesis, with a hypothesis. *BJU Int* 2000;85:995–9.
4. Saban R, Saban MR, Maier J, et al. Urothelial expression of neuropilins and VEGF receptors in control and interstitial cystitis patients. *Am J Physiol Renal Physiol* 2008;295: F1613–23.
5. Southgate J, Varley CL, Garthwaite MA, et al. Differentiation potential of urothelium from patients with benign bladder dysfunction. *BJU Int* 2007;99:1506–16.
6. Parsons CL. The role of the urinary epithelium in the pathogenesis of interstitial cystitis/prostatitis/urethritis. *Urology* 2007;69(Suppl 4):9–16.
7. Soler R, Bruschini H, Freire MP, Alves MT, Srouqi M, Ortiz V. Urine is necessary to provoke bladder inflammation in protamine sulfate induced urothelial injury. *J Urol* 2008; 180:1527–31.
8. Teichman JM, Moldwin R. The role of the bladder surface in interstitial cystitis/painful bladder syndrome. *J Urol* 2007;14:3599–607.
9. Neuhaus J, Heinrich M, Schlichting N, et al. Structure and function of suburothelial myofibroblasts in the human urinary bladder under normal and pathological conditions. *Urologe A* 2007;46:1197–202.
10. Steers WD, Tuttle JB. Mechanisms of disease: the role of nerve growth factor in the pathophysiology of bladder disorders. *Nat Clin Pract Urol* 2006;3:101–10.
11. Cui M, Khanijou S, Rubino J, et al. Subcutaneous administration of botulinum toxin A reduces formalin-induced pain. *Pain* 2004;107:125–33.
12. Seki S, Sasaki K, Fraser MO, et al. Immunoneutralization of nerve growth factor in lumbosacral spinal cord reduces bladder hyperreflexia in spinal cord injured rats. *J Urol* 2002;168:2269–74.
13. Chancellor MB, Yoshimura N. Treatment of interstitial cystitis. *Urology* 2004;63(Suppl 1):85–92.
14. Dodd LG, Tello J. Cytologic examination of urine from patients with interstitial cystitis. *Acta Cytol* 1998;42:923–7.
15. Bouchelouche K, Kristensen B, Nordling J, Horn T, Bouchelouche P. Increased urinary leukotriene E4 and eosinophil protein X excretion in patients with interstitial cystitis. *J Urol* 2001;166:2121–5.
16. Sun Y, Chai TC. Augmented extracellular ATP signaling in bladder urothelial cells from patients with interstitial cystitis. *Am J Physiol Cell Physiol* 2006;290:C27–34.
17. Sun Y, Chen M, Lowentritt BH, et al. EGF and HB-EGF modulate inward potassium current in human bladder urothelial cells from normal and interstitial cystitis patients. *Am J Physiol Cell Physiol* 2007;292:C106–14.
18. Zhang CO, Wang JY, Koch KR, Keay S. Regulation of tight junction proteins and bladder epithelial paracellular permeability by an antiproliferative factor from patients with interstitial cystitis. *J Urol* 2005;174:2382–7.

19. Keay SK, Zhang CO, Shoenfelt J, et al. Sensitivity and specificity of antiproliferative factor, heparin-binding epidermal growth factor-like growth factor, and epidermal growth factor as urine markers for interstitial cystitis. *Urology* 2001;57(Suppl 1):9–14.
20. Chai TC, Zhang CO, Shoenfelt JL, Johnson HW Jr, Warren JW, Keay S. Bladder stretch alters urinary heparin-binding epidermal growth factor and antiproliferative factor in patients with interstitial cystitis. *J Urol* 2000;163:1440–4.
21. Kim J, Keay SK, Dimitrakov JD, Freeman MR. p53 mediates interstitial cystitis antiproliferative factor (APF)-induced growth inhibition of human urothelial cells. *FEBS Lett* 2007;581:3795–9.
22. Keay S, Kleinberg M, Zhang CO, Hise MK, Warren JW. Bladder epithelial cells from patients with interstitial cystitis produce an inhibitor of heparin-binding epidermal growth factor-like growth factor production. *J Urol* 2000;164:2112–8.
23. Graham E, Tsai TC. Dysfunction of bladder urothelium and bladder urothelial cells in interstitial cystitis. *Curr Urol Rep* 2006;7:440–6.
24. Slobodov G, Feloney M, Gran C, et al. Abnormal expression of molecular markers for bladder impermeability and differentiation in the urothelium of patients with interstitial cystitis. *J Urol* 2004;171:1554–8.
25. Schonk DM, Kuijpers HJ, van Drunen E, et al. Assignment of the gene(s) involved in the expression of the proliferation-related Ki-67 antigen to human chromosome 10. *Hum Genet* 1989;83:297–9.
26. Hauser PJ, Dozmorov MG, Bane BL, Slobodov G, Culkin DJ, Hurst RE. Abnormal expression of differentiation related proteins and proteoglycan core proteins in the urothelium of patients with interstitial cystitis. *J Urol* 2008;179:764–9.
27. Hurst RE, Moldwin RM, Mulholland SG. Bladder defense molecules, urothelial differentiation, urinary biomarkers, and interstitial cystitis. *Urology* 2007;69:17–23.
28. Green M, Filippou A, Sant G, Theoharides TC. Expression of intercellular adhesion molecules in the bladder of patients with interstitial cystitis. *Urology* 2004;63:688–93.
29. Leppilahti M, Hellstrom P, Tammela TL. Effect of diagnostic hydrodistention and four intravesical hyaluronic acid instillations on bladder ICAM-1 intensity and association of ICAM-1 intensity with clinical response in patients with interstitial cystitis. *Urology* 2002;60:46–51.
30. Yamada T, Nishimura M, Mita H. Increased number of apoptotic endothelial cells in bladder of interstitial cystitis patients. *World J Urol* 2007;25:407–13.
31. Keay S, Zhang CO, Shoenfelt JL, Chai TC. Decreased *in vitro* proliferation of bladder epithelial cells from patients with interstitial cystitis. *Urology* 2003;61:1278–84.
32. Keay S. Cell signaling in interstitial cystitis/painful bladder syndrome. *Cell Signal* 2008;20:2174–9.
33. Murray E, Malley SE, Qiao LY, Hu VY, Vizzard MA. Cyclophosphamide induced cystitis alters neurotrophin and receptor tyrosine kinase expression in pelvic ganglia and bladder. *J Urol* 2004;172:2434–9.
34. Oddiah D, Anand P, McMahon SB, Rattray M. Rapid increase of NGF, BDNF and NT-3 mRNAs in inflamed bladder. *Neuroreport* 1998;9:1455–8.
35. Chung YC, Fraser MO, Yu Y, Chancellor MB, de Groat WC, Yoshimura N. The role of bladder afferent pathways in bladder hyperactivity induced by the intravesical administration of nerve growth factor. *J Urol* 2001;165:975–9.
36. Lamb K, Gebhart GF, Bielefeldt K. Increased nerve growth factor expression triggers bladder overactivity. *J Pain* 2004;5:150–6.
37. Kuo HC. Preliminary results of suburothelial injection of botulinum A toxin in the treatment of chronic interstitial cystitis. *Urol Int* 2005;75:170–4.
38. Giannantoni A, Costantini E, Di Stasi SM, Tascini MC, Bini V, Porena M. Botulinum A toxin intravesical injections in the treatment of painful bladder syndrome: A pilot study. *Eur Urol* 2006;49:704–49.
39. Liu HT, Kuo HC. Intravesical botulinum toxin A injections plus hydrodistension can reduce nerve growth factor production and control bladder pain in interstitial cystitis. *Urology* 2007;70:463–8.