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Urinary Bladder, Cystitis and Nerve/Urothelial Interactions

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Abstract

A hallmark of functional pain syndromes, such as bladder pain syndrome/interstitial cystitis (BPS/IC) is pain in the absence of demonstrable infection or pathology of the viscera or associated nerves. There are no clear definitions of this syndrome, no proven etiologies and no effective treatments able to eradicate the symptoms. This condition is characterized by suprapubic pain, associated with bladder filling and can also be accompanied by a persistent strong desire to void, increased frequency of urination and nocturia. Severe cases of this disorder, which affects primarily women, can have considerable impact on the quality of life of patients due to extreme pain and urinary frequency, which are often difficult to treat. In addition, BPS/IC patients may also suffer co-morbid conditions where pain is a common symptom (such as irritable bowel syndrome, fibromyalgia). Theories explaining the pathology of bladder pain syndrome are many and include an altered bladder lining and possible contribution of a bacterial agent.

1.0 Introduction

Bladder pain syndrome/interstitial cystitis (BPS/IC) is a debilitating chronic disease characterized by suprapubic pain related to bladder filling, coupled with additional symptoms, such as increased day- and night-time urinary frequency, without proven urinary infection or other obvious pathology. Although the symptoms presented may appear similar to those of a urinary tract infection, urine culture reveals no underlying infection and there is no response to antibiotic treatment (Parsons et al., 1993; Hanno et al., 1999; Bladder Research Progress Review Group, 2002). Between 700,000 and 1 million people in the United States have IC, the preponderance of who are women (Bladder Research Progress Review Group, 2002). Moreover, it has been estimated that a 60% increase in the number of cases would be identified by experienced clinicians who apply the strict National Institute of Diabetes, Digestive, and Kidney Diseases definition of BPS/IC (Hanno et al., 1999). While the etiology is unknown, theories explaining the pathology of BPS/IC include altered barrier

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lining, afferent and/or CNS abnormalities, possible contribution of inflammatory or bacterial agent and abnormal urothelial signaling.

2.0 Disease Process and Relevant Animal Models

The etiology of BPS/IC is unknown; however, several causes have been postulated, including epithelial dysfunction (i.e., leaky urothelium), infection, autoimmune response, allergic reaction, neurogenic inflammation, and inherited susceptibility (Bladder Research Progress Review Group, 2002; NIH Publication No. 02-3220, 2002).

A number of animal models have been used for the study of BPS/IC, which includes administration of an irritant or immune stimulant (e.g. hydrochloric acid, turpentine, protamine sulfate, mustard oil, lipopolysaccharide and cyclophosphamide). Studies have shown that deficiency of estrogen receptor-beta in female mice develop a bladder phenotype (including alterations in the urothelium) which may share similarities with human PBS/IC (Imamov et al., 2007). However, a review of such animal models discusses the potential problems in artificially inducing bladder inflammation or injury and thus may not be considered a valid method to model the symptoms of this complex syndrome (Westropp, 2002; Buffington, 2008). Furthermore, the degree of bladder hyperreflexia observed in rodents is variable and can resolve within a matter of days. This may be, in part, due to the capacity of the damaged rodent bladder urothelium to rapidly regenerate post-intravesical insult thus limiting the capacity to establish chronicity in these models reflective of the human condition.

A naturally occurring disease occurring in cats, termed feline interstitial cystitis, reproduces many features of BPS/IC in humans diagnosed with this disorder (Buffington, 2008). In addition, an experimental autoimmune cystitis (EAC) murine model has been shown to exhibit a number of comparable functional and histological alterations to that in human BPS/IC (Line et al., 2008). Also similar to BPS/IC patients, pseudorabies virus (PRV) injection in mice results in the development of a neurogenic cystitis associated with pelvic pain and accumulation of mast cells (Rudick et al., 2009). Stress has been shown to impair the immune, endocrine and nervous systems and can be an important factor in functional gastrointestinal (GI) and genitourinary (GU) disorders such as irritable bowel syndrome (IBS) and BPS/IC. For example, rats exposed to various types of stress (water avoidance, intruder stress) exhibit symptoms of bladder dysfunction including increased micturition frequency as well as anxiety-like behavior (Smith et al., 2008; Wood et al 2009). Further, an exaggerated acoustic startle response has been demonstrated in both cats diagnosed with feline IC as well as in BPS/IC patients (Westropp and Buffington, 2006; Twiss et al., 2009). This response is a brainstem reflex responding to unexpected loud stimuli and parallels that of autonomic control. Even though the pathophysiology and etiology of most persistent pain syndromes are incompletely understood, it is generally assumed that they involve changes in the target organ as well as alterations in both central and peripheral processing/modulation of nociception and pain. In addition, while alterations in the periphery may alter nociceptive input to the CNS, pain remains an emergent property of the brain. A number of recent studies have identified structural and functional changes in the brain of patients with chronic

pain syndromes that may influence the perception of sensory input (May, 2008; Mayer and Bushnell, 2009)

Due to the complex nature of BPS/IC it is thus unlikely that a single animal model would be suitable for investigative work and thus a panel of models reflecting the key symptoms and known components of the condition should be utilized to investigate potential therapeutic mechanisms.

2.1 Epithelial Alterations

The urothelium, the epithelial lining of the urinary tract between the renal pelvis and urinary bladder, is composed of at least three cell layers (see Figure 1A). These consist of a basal cell layer, an intermediate and a superficial or apical layer composed of cells termed “umbrella” cells, which are interconnected by tight junctions. Apical urothelial cells function as a barrier against most substances found in urine thus protecting the underlying tissues (Negrete et al., 1996; Zeidel, 1996; Lewis, 2000; Apodaca, 2004). When this function is compromised during injury or inflammation, it can result in the passage of toxic substances into the underlying tissue (neural/muscle layers) resulting in urgency, frequency and pain during voiding. The superficial umbrella cells play a prominent role in maintaining this barrier, and exhibit a number of properties including specialized membrane lipids, asymmetric unit membrane particles and a plasmalemma with stiff plaques (Lewis, 2000; Hu et al., 2002; Apodaca, 2004).

BPS/IC has often been described as a disease of the urothelium (Graham and Chai, 2006). Ultrastructurally, an altered vascular supply is observed in its ulcerative form with locations of moderate-to-severe redness, interspersed among a whitish discoloration. There is also evidence that the urothelium in BPS/IC is associated with altered synthesis of a number of proteins including those involved in cellular differentiation, barrier function and bacterial defense mechanisms. In this regard, studies have shown in patients diagnosed with the ulcerative form of BPS/IC, that laser removal of damaged urothelium is associated with reduction of symptoms of bladder or pelvic pain (Rofeim et al., 2001). This treatment stimulates a rapid urothelial turnover and patients undergoing this treatment report a prolonged period without pain (6-12 months) after therapy. In terms of barrier ‘repair’, instillation of liposomes composed of phospholipids has been shown to support repair of the urothelial barrier in animals following bladder irritation (Tyagi et al., 2009). Though the mechanism is not well defined, by forming a protective coating on the urothelium, liposomes may act as a mucosal protective agent and thereby decreasing irritation of underlying afferent nerves. In this regard use of intravesical liposomes for treatment of patients with ulcerative BPS/IC has shown promise to repair and enhance the barrier function of a dysfunctional urothelium though further trials are needed to fully assess this type of treatment (Peters et al., 2012).

Disruption of the integrity of the urothelial barrier may be mediated by hormonal or neural mechanisms (such as by substances released by surrounding afferent and autonomic nerve fibers and various cell types within the bladder wall such as immune or inflammatory cells). For example, nitric oxide (NO) has been demonstrated to be a marker for inflammatory bladder disorders. In addition, nitric oxide (NO) was elevated in patients with interstitial

cystitis as well as cats diagnosed with feline interstitial cystitis (Hosseini et al., 2004; Birder et al., 2005). BPS/IC patients diagnosed with a Hunner lesion (areas of inflammation on the bladder wall that characterize the classic form of BPS/IC) exhibited high intraluminal levels of NO as well as inflammatory infiltrates within the urothelium and also lamina propria (Logadottir et al., 2013). Excessive NO levels in the urinary bladder can increase permeability of the urothelium to water/urea in addition to producing ultrastructural changes in the apical layer. The NO can be synthesized/released by a number of cell types within the bladder wall (including the urothelium) and, after release can act in an autocrine/paracrine manner to alter urothelial permeability to ions and solutes (see Fig. 1B). Although the pathological mechanism is unknown, these findings appear to be similar to that in other epithelia where excess production of NO has been linked to changes in epithelial integrity. Disruption of epithelial integrity may also be linked to expression of substances such as antiproliferative factor (APF), which has been characterized as a frizzled-8-related sialoglycopeptide and is detected in the urine of patients with bladder pain syndrome (Keay et al., 2001). Abnormalities in urothelial growth and proliferation may also be linked to changes in expression of trophic factors such as HB-EGF.

Changes in epithelial signaling/barrier function are not unique to the urinary bladder. For example, airway epithelia in asthmatic patients as well as keratinocytes in certain types of skin disease also exhibit a number of similar abnormalities and compromised repair processes (Bosse et al., 2008). Taken together, epithelial cells can respond to a number of challenges (including environmental pollutants and mediators released from nerves or nearby inflammatory cells) resulting in altered expression and/or sensitivity of various receptor/channels as well as changes in release of mediators, which may impact function.

2.2 GAG layers

The urothelial surface is lined by surface mucin, a heterogeneous, gel-like substance composed of numerous sulfonated glycosaminoglycans (GAG) and glycoproteins. Many clinicians believe that a defect in the protective GAG layer of the bladder that lines the epithelium of the bladder is responsible for the permeability changes of the bladder in BPS/IC. However, the urothelial barrier function of the GAG layer has been a controversial subject. Lilly and Parsons report that intravesical treatment of the rabbit bladder with protamine sulfate increased urothelial permeability to water, urea and calcium both *in vivo* and *in vitro* (Lilly and Parsons, 1990). This effect was reversed with pentosanpolysulfate (PPS) (Lilly and Parsons, 1990). They concluded that the protamine sulfate affected the GAG layer and that this was repaired by PPS. However no microscopic evidence of the anatomical changes was presented in this paper. This study was later confirmed by Nickel et al. (1998) who compared PPS, heparin and hyaluronic acid as treatments. The authors concluded that heparin was the best of the three agents in efficacy, but pointed out that this may be due to its anti-inflammatory properties. Indeed the role of the GAG layer may be more in line with an antibacterial adherence function (Hanno et al., 1981). The GAG layer may also be important for the formation and attachment of particulates to the urothelium and stone formation (Grases 1966; Hurst 1994).

Overall, it seems that protamine sulfate does not act at the level of the GAG layer but rather at the surface of the luminal cells, so called “umbrella cells” (Fig. 1A), thus enhancing urea absorption by other mechanisms. Protamine sulfate increases the permeability of the apical membrane (luminal surface of umbrella cells) permeability to both monovalent cations and anions. This effect may be reversible depending on the concentration of the protamine, the composition of the bathing solution and the exposure time of the urothelium to protamine. Prolonged exposure to protamine (>15minutes) is poorly reversible and is thought to be due to a decrease in paracellular resistance due to cell lysis (Tzan et al., 1993; 1994).

In summary the GAG layer may have importance in bacterial anti-adherence, and prevention of urothelial damage by large macromolecules. However, there is no definite evidence that the GAG layer acts as the primary epithelial barrier between urine and plasma.

3.0 Protective Functions

3.1 Importance of the autonomic nervous system

Another characteristic change observed in many types of epithelia following injury, and in the urothelium following a spinal cord injury, is an early degeneration followed by regeneration. In rodents, it has been shown that 2 hours after experimental transection of the spinal cord, there is a significant disruption of the urothelial barrier with accompanying permeability and ultrastructural changes (Apodaca et al., 2003). These alterations are prevented by pretreatment with ganglionic blockers, suggesting that the autonomic (parasympathetic or sympathetic) innervation play a role in acute response of urothelial cells to spinal cord injury (Fig. 1A). The mechanism underlying the neuronally induced changes of the urothelium is unknown. There is limited understanding of tight junction assembly or turnover, however, when the urothelium is injured by a transection of the spinal cord or exposure to uropathogenic *Escherichia coli*, it undergoes rapid regeneration after disruption of the urothelial barrier. Epithelial integrity is maintained through a complex process of proliferation and differentiation. During a regenerative process, studies suggest that proliferation takes precedence over differentiation.

3.2 Immune-epithelial interactions and cellular defense

Both physiological and psychological stress can result in a failure of urothelial and suburothelial ‘defensive’ systems and thereby promote changes in both urothelial barrier and signaling function. Epithelia are also able to secrete a number of antimicrobial molecules such as Tamm-Horsfall protein (THP), cytokines, and defensins, which are small peptides thought to have antimicrobial activity (Ganz, 2001; Zasloff, 2002). The defensins, which are abundant in epithelia of the intestine, respiratory and urinary tract, are also thought to exhibit additional functions including regulation of inflammation and in the adaptive immune response. Alterations in proteins including proteoglycans and bacterial defense molecules may lead to distinctive changes in urothelial structure and play a role in bacterial adherence (Rostand and Esko, 1997). In addition, toll-like receptors (TLRs), which are a family of membrane glycoproteins, also play an important role in the initiation of immune responses in various types of epithelium, such as the airways and both gastrointestinal and genitourinary tracts (Weichhart et al., 2008; Kumar et al., 2009; Jager et al., 2013). Although

the urothelium maintains a tight barrier, a number of factors (e.g. mechanical or chemical trauma, infection) can modulate the barrier function. When the barrier is compromised, the urothelium is unable to maintain the integrity of the bladder-urine interface. The result can be changes in the function of urothelial cells and terminals of visceral afferent neurons within the bladder wall resulting in symptoms of urgency, frequency and pain during bladder filling and voiding. Thus, a complex chemical information transfer exists between the urothelium and cells within the bladder wall (which can include neuroendocrine, immune and autonomic nervous systems, see Figure 1A) and disruption in this ‘sensory web’ may be involved in bladder dysfunction.

4.0 “Neuron-like” Properties of the Urothelium

While the urothelium has been historically viewed as primarily a “barrier”, it is becoming increasingly appreciated as a responsive structure capable of detecting physiological and chemical stimuli, and releasing a number of signaling molecules (see Figure 1B). Data accumulated over the last several years now indicate that urothelial cells display a number of properties similar to sensory neurons (nociceptors/mechanoreceptors), involving diverse signal-transduction mechanisms to detect physiological stimuli. Examples of “sensor molecules” (i.e. receptors/ion channels) associated with neurons that have been identified in urothelium. These include receptors for bradykinin (Chopra et al., 2005) neurotrophins (trkA and p75) (Murray et al., 2004; Birder et al., 2010), purines (P2X and P2Y) (Lee et al., 2000; Burnstock, 2001; Hu et al., 2002; Birder et al., 2004; Tempest et al., 2004; Wang et al., 2005), norepinephrine (α and β) (Birder et al., 1998; Birder et al., 2002a), acetylcholine (nicotinic and muscarinic) (Chess-Williams, 2002; Beckel et al., 2006; Beckel and Birder, 2012), protease activated receptors (PARs) (D’Andrea et al., 2003), amiloride/mechanosensitive Na^+ channels (Lewis and Hanrahan, 1985; Lewis, 1991; Smith et al., 1998; Carattino et al., 2005) and a number of TRP channels (TRPV1, TRPV2, TRPV4, TRPM8) (Birder et al., 2001, 2002b; Stein et al., 2004).

5.0 What is the Source of Pain in BPS/IC?

Chronic pain conditions such as bladder pain syndrome are associated with changes in the central nervous system as well as the periphery (Berkley, 2005). While the etiology or source of pain is unknown, the relationship is likely to be complicated, involving both abnormalities in central pain processing as well as changes in the target organ.

The urothelium is likely to play an important role by actively communicating with bladder nerves (in particular afferent neurons), smooth muscle cells, lamina propria interstitial cells and cells belonging to the immune and inflammatory systems. Altered expression or sensitivity of molecular targets such as TRPV1, acid-sensing channels and muscarinic receptors have been reported in BPS/IC patients as well as in animal models for the syndrome (Cruz and Denis, 2007; Gupta et al., 2009; Ikeda et al., 2009; Sanchez-Freire et al., 2011). In addition, augmented release of transmitters, most notably ATP, from the urothelium can lead to painful sensations by excitation of purinergic receptors on sensory fibers (Sun and Chai, 2006; Birder et al., 2010; Burnstock, 2012). Thus, inhibition of purinergic P2X3 receptors on afferent terminals (Fib. 1B) has been shown to be effective in

suppressing afferent excitation in various animal models and may be effective in clinical conditions associated with pain such as BPS/IC (Gever et al., 2010; Ford, 2012). Onabotulinum toxin A (BoNT-A) has been used in the treatment of lower urinary tract disorders including BPS/IC and appears to have a positive therapeutic effect (Chancellor et al., 2008). By inhibiting SNARE-dependent exocytotic processes, BoNT-A can prevent the release of transmitters (such as ATP) as well as normalizing the expression of various receptors, channels and trophic factors (Liu and Kuo, 2007). Studies have shown that BoNT/A treatment normalized changes in urothelial receptor expression and neurotransmitter levels in animals with experimental bladder overactivity and in patients diagnosed with detrusor overactivity (Smith et al., 2008). These and other studies suggest that the urothelium may be a target for this treatment and that urothelial-released mediators may contribute to sensory urgency/pain by activating or sensitizing visceral afferents innervating the urinary bladder.

6.0 Can we identify a ‘biomarker’?

There is a great deal of interest in identifying a ‘biomarker’ that could be of value in the diagnosis (and not just predictive of symptom progression) for BPS/IC. A range of factors have been studied including APF, epidermal growth factor (EGF), insulin-like growth factor 1 (IGF1), insulin-like growth factor binding protein 3 (IGFBP3) as well as urinary chemokines and have been shown to be correlated to BPS/IC. While some reports suggest urinary markers (e.g., APF, HB-EGF; EGF) may be useful to discriminate BPS/IC and asymptomatic controls, (Keay et al., 2004; Zhang et al., 2005), these may not correlate with findings using bladder biopsies (Erickson et al., 2008). The reason in part may be due to variability in biopsy location, depth of sample (containing different cell types) as well as other technical variations. There is some suggestion that there seems to be more of a proinflammatory state with increased infiltration of mast cells in BPS/IC patients as compared to controls. In addition, increased levels of NGF in urine and tissue have been linked with bladder pathologies including patients with idiopathic “sensory urgency (urgency without incontinence), overactivity, and BPS/IC (Liu and Kuo., 2007; Kuo et al., 2010). Studies in cats diagnosed feline IC have reported increased NGF levels in bladder urothelium (Birder et al., 2010) and a major source of NGF has been shown to come from urothelium, which may contribute to increased neural excitability and emergence of bladder pain in BPS/IC (Micera et al., 2007).

7.0 Summary

In summary, these findings suggest that urothelial cells exhibit specialized sensory and signaling properties that could allow them to respond to their chemical and physical environments and to engage in reciprocal communication with neighboring urothelial cells as well as nerves within the bladder wall. Taken together, pharmacologic interventions aimed at targeting urothelial receptor/ion channel expression or transmitter release mechanisms may provide a new strategy for the clinical management of bladder disorders such as BPS/IC.

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Figure 1.

A) Cartoon depicting various components of the bladder wall. These include subtypes of urothelial cells (with apical or umbrella cells connected via tight junctions), layers of smooth muscle cells; interstitial cells (in green) whose functions have not yet been defined but may play a role in intercellular communication, sacral afferent innervation (blue), and autonomic (parasympathetic and sympathetic) innervation (red). Pre, preganglionic; post, postganglionic. B) Hypothetical model depicting possible interactions between afferent nerve fibers (blue), urothelial cells, smooth muscle and interstitial cells. Urothelial cells can also be targets for transmitters (such as ATP, nitric oxide-NO, acetylcholine-ACh) released from nerves or other cell types. Urothelial cells can be activated by either autocrine (i.e. autoregulation) or paracrine (release from nearby nerves or other cells) mechanisms. For example, mechanical stimuli such as bladder distension can release urothelial-acetylcholine, which then activates urothelial cholinergic (muscarinic) receptor subtypes, resulting in release of additional transmitters. M3-muscarinic subtype receptor; P2R or P2X/P2Y-purinergic subtype receptors; GC-guanylate cyclase; IP₃-inositol triphosphate; PKC-protein kinase C; PKG-cGMP-dependent protein kinase; Ca²⁺-calcium; TRPV- transient receptor potential family of ion channels; NOS- nitric oxide synthase.