Purinergic P2X7 receptors as therapeutic targets in interstitial cystitis/bladder pain syndrome; key role of ATP signaling in inflammation

Zhinoos Taidi[1], Kylie J. Mansfield[2], Lucy Bates[3], Hafiz Sana-Ur-Rehman[1], Lu Liu[1]*

[1]School of Medical Sciences, The University of New South Wales, Sydney NSW 2052, Australia
[2]School of Medicine, University of Wollongong, Wollongong, NSW 2522, Australia
[3]Westmead Hospital, Westmead, NSW 2145, Australia

*Corresponding author: Lu Liu, Email: lu.liu@unsw.edu.au

ABSTRACT

Interstitial cystitis/bladder pain syndrome (IC/BPS) is a chronic lower urinary tract condition. Patients with IC/BPS suffer from debilitating pain and urinary urgency. The underlying etiology of IC/BPS is unknown and as such current treatments are mostly symptomatic with no real cure. Many theories have been proposed to describe the etiology of IC/BPS, but this review focuses on the role of inflammation. In IC/BPS patients, the permeability of the urothelium barrier is compromised and inflammatory cells infiltrate the bladder wall. There are increased levels of many inflammatory mediators in patients with IC/BPS and symptoms such as pain and urgency that have been associated with the degree of inflammation. Recent evidence has highlighted the role of purinergic receptors, specifically the P2X7 receptor, in the process of inflammation. The results from studies in animals including cyclophosphamide-induced hemorrhagic cystitis strongly support the role of P2X7 receptors in inflammation. Furthermore, the deletion of the P2X7 receptor or antagonism of this receptor significantly reduces inflammatory mediator release from the bladder and improves symptoms. Research results from IC/BPS patients and animal models of IC/BPS strongly support the crucial role of inflammation in the pathophysiology of this painful disease. Purinergic signaling and purinergic receptors, especially the P2X7 receptor, play an undisputed role in inflammation. Purinergic receptor antagonists show positive results in treating different symptoms of IC/BPS.

Keywords: ATP, bladder pain syndrome, inflammation, interstitial cystitis, purinergic receptors

INTRODUCTION

Interstitial cystitis (IC) is a chronic lower urinary tract condition. IC is also called bladder pain syndrome (BPS) [1] and was previously referred to as painful bladder syndrome (PBS) [2]. All three terms are considered to refer to the same condition. The term "IC/BPS" has been widely used in the literature and will be used here. The underlying etiology of IC/BPS is unknown and as such the current therapeutic approaches are mostly symptomatic with no real cure available. To date, many theories have been proposed to describe the etiology of IC/BPS, but this review focuses on the role of inflammation in this disease. Since Burnstock introduced purinergic signaling in the 1970s, many different roles of purinergic receptors have been revealed but one of the most recent major topics is their role in the process of inflammation. Thus, this review will discuss the possible involvement of purinergic receptors and ATP as their signaling molecule in the pathophysiological development of IC/BPS with particular focus on the P2X7 receptor, given that recent studies have revealed that P2X7 receptor is a potent stimulant of inflammation and immune response, which makes this receptor a potential target for anti-inflammatory treatment.

INTERSTITIAL CYSTITIS/BLADDER PAIN SYNDROME

IC/BPS is a chronic lower urinary tract condition characterized by pain perceived to be associated with the bladder filling [3]. The main

symptoms associated with IC/BPS include pain on bladder filling which is often related to urinary urgency, a sudden compelling desire to void urine, as well as nocturia, waking at night to void, which can cause sleep disturbance in these patients [4]. These are usually accompanied by other symptoms, such as a dramatic decrease in bladder capacity and dyspareunia; pain during sexual intercourse [5]. The decrease in bladder capacity leads to an increased urinary frequency which is also due to the pain associated with bladder filling. The etiology of IC/BPS is unknown. Therefore IC/BPS is not easily diagnosed, especially in the early stages as the symptoms of urgency, frequency and nocturia can be similar to other urological diseases such as overactive bladder or urinary tract infections [6]. Evidence of inflammation in biopsy samples from IC/BPS patients is part of the diagnosis of the disease although routine biopsies are not recommended for IC/BPS patients [1]. Based on the guidelines of American Urological Association and Canadian Urological Association, there is no urinary tract infection (UTI) at the time of diagnosis but up to 50% of IC/BPS patients might have a history of previous UTI [1].

CURRENT TREATMENT OPTIONS

The current therapeutic approaches for IC/BPS patients are mostly symptomatic with no real cure identified [1]. The current treatments include behavioral therapy, oral medications, intravesical treatments as well as surgical treatments. Behavioral therapy involves modifying daily fluid intake, clothes, food and sexual behavior [7,8] with the aim of reducing the likelihood of triggering symptoms. In terms of oral medications, a variety of different agents have been used with variable success in IC/BPS patients. These include the tricyclic antidepressant amitriptyline, antihistamine/anti-anxiety medication hydroxyzine, and as a last resort, the immunosuppressant agent cyclosporine A and also pentosan polysulfate sodium (PPS), which forms a protective layer on the bladder lining [9-13]. In addition to these oral medications, dimethyl sulfoxide (DMSO), heparin and lidocaine have been used topically to decrease pain by instilling into the bladder lumen in IC/BPS patients either separately or in combination [14-17]. Intravesical therapy by injecting botulinum toxin A (BTX-A) into the bladder wall of IC/BPS patients has shown some improvement in pain, voiding frequency and patients' quality of life [18-21].

The effectiveness of all these therapies, regardless of being systemic or topical, is still under consideration. A recent meta-analysis on intravesical therapy for IC/BPS concluded that there are insufficient well-controlled clinical trials to provide scientific evidence of efficacy [22]. The clinical trials that have been conducted have demonstrated fairly low response rates, with regard to improvement in bladder pain and urgency, for the available therapies. For example, two clinical trials of intravesical PPS in IC/BPS patients showed 34% [23] and 40% response rates [24], while intravesical DMSO reported response rates of 30% [25] and 14% [26], which are not significantly different from the placebo response rate of ~20% [23]. These studies demonstrate that the effectiveness of the currently available therapies is low and there is a need for the development of new and effective treatment options.

CLINICAL EVIDENCE OF INFLAMMATION IN IC/BPS

Observations from cystoscopy and biopsy samples from IC/BPS patients

The importance of inflammation in the pathophysiology of IC/BPS can be clearly seen on cystoscopy. The most common finding on cystoscopy of patients with IC/BPS is mucosal damage and hemorrhages [27]. IC/BPS is diagnosed at cystoscopy when the bladder has been filled to its maximum capacity (at a pressure of 80–100 cm H_2O). In IC/BPS patients mucosal splitting or glomerulations (petechial hemorrhages) are seen in a minimum of 3 quadrants, with 10 per quadrant. It should be noted that two types of IC/BPS have been defined based on cystoscopy findings, that are: IC/BPS with or without Hunner ulcer [28]. Hunner ulcers, visualized on cystoscopy, are areas away from the bladder trigone that are either inflamed areas of the mucosa that may have small vessels joining each other at one central point, or may appear pale, non-blanching if in the chronic state. These ulcers can easily split open with bladder distention which causes bleeding [28]. The presence or absence of Hunner ulcer in IC/BPS patients is believed to have an important role in symptom variations, differences in therapeutic success and the level of pain, especially the pain related to bladder distension [28-30]. Mucosal splitting, glomerulations and Hunner ulcers are all signs of mucosal damage.

The role of inflammation in mucosal damage is evident in biopsies collected from IC/BPS patients. Recent studies have emphasized inflammation as the common cause of different types of urinary tract dysfunction [31]. In biopsies collected from IC/BPS patients, it has been shown that inflammation is associated with disruption to the urothelial cell layer [32-35], increased density of sub-urothelial afferent nerve fibers [36], and infiltration of inflammatory cells such as mast cells [37-39]. However, it is unknown whether inflammation causes damage to the mucosa or it is the consequence of mucosal damage.

Urothelial changes

There are many changes to the urothelial cells in patients with IC/BPS. The urothelium of IC/BPS patients is thinner, usually only 1 or 2 cells thick, compared to the urothelium from normal bladders which is approximately 5 cells thick [40]. Also in IC/BPS patients, the urothelium does not include a distinct layer of umbrella cells [40] and urothelial cell apoptosis has been described [32]. These findings suggest that disruption to the structure of the urothelium is a feature of the pathophysiology of IC/BPS. Associated with the changes in the urothelial structure is the disruption to the permeability function of the urothelium. Although the main role of bladder urothelium is thought to provide a barrier which is impermeable to all urinary solutes, reduced urothelial barrier function has been observed in patients with IC/BPS [40,41]. A recent study in rats has shown altered expression and function of paracellular tight junctions in the urothelium may trigger bladder inflammation and promote cystitis [42].

On the luminal side of the bladder wall, a glycosaminoglycan (GAG) layer, a highly hydrated barrier surface, acts as the first protecting layer against urinary toxins and solutes. This GAG layer is made up of hyaluronate, chondroitin sulfate, heparin sulfate, keratin sulfate and dermatan sulfate [43]. Deficiency of the luminal and basal chondroitin sulfate proteoglycan layers has been demonstrated in bladder biopsies from IC/BPS patients, and therefore it was proposed that GAG layer deficiencies may be associated with permeability changes in IC/BPS

patients [44,45]. However, other studies have shown that although GAG layer can be a barrier for the movement of microorganisms, its contribution to the urothelial permeability is unclear [46,47] (for reviews see [48,49]).

GAG layer replenishment is one of the main therapeutic strategies in the treatment of IC/BPS. The aim of the intravesical instillations of PPS, DMSO and heparin is to promote the recovery of the damaged GAG layer. PPS, which is similar in structure to the natural GAG, is believed to replenish the protective covering of the urothelium. DMSO is thought to reduce inflammation and act as an analgesic and heparin, which is a sulfonated GAG, has both anti-inflammatory and surface replenishing properties [50]. However, as mentioned previously, the effectiveness of these two therapies is low [24,26], which may be explained by the observations that the GAG layer has no influence on urothelial permeability [46,47].

Other urothelial associated proteins also contribute to the permeability barrier. These include the uroplakins that form a covering over the urothelial luminal surface [51] and the tight junction proteins that bind the urothelial cells together [42]. In patients with IC/BPS, there is an absence of the tight junction protein zona occludens-1 (ZO-1), and also there are deficiencies in the expression of uroplakin and E-cadherin [40]. The change in ZO-1 expression may be associated with the increased permeability of the bladder urothelium in IC/BPS patients [41]. It is highly likely that both the decreased thickness of the urothelial cell layer and the increased urothelial permeability are associated with the symptoms that patients experience such as pain and urgency. Both of these symptoms have negative effects on the quality of life in these patients.

Increased inflammatory mediators

The core role of inflammation in the etiology of IC/BPS has been investigated in many studies. The initial evidence for inflammation in biopsies from patients with IC/BPS was increased mast cell counts. The increase in mast cells in biopsies from patients with IC/BPS compared to normal control was described in the detrusor muscle layer [5] and also in the sub-urothelial layer [52,53]. Interestingly, the increased number of mast cells in the sub-urothelial layer was localized to the areas underlying ulcers in patients with IC/BPS [52]. This same study showing localized mast cells within the urothelial layer has also demonstrated the presence of mast cells in the intravesical fluid [52].

Higher counts of other inflammatory cells such as neutrophils and eosinophils have been also reported in the urine samples from IC/BPS patients [54]. Neutrophils make up approximately 70% of white blood cells and have a well-known contribution to the acute inflammatory response [55]. Eosinophils, on the other hand, are important regulators of local inflammatory response and their accumulation is linked to many inflammatory diseases [56]. Many studies have linked the damaged urothelium to activation and stimulation of the inflammatory cells in the bladder of IC/BPS patients which can trigger the release of other inflammatory mediators [57-61].

In IC/BPS patients, there is also an increase in the concentration of inflammatory mediators such as histamine, which is released following mast cell activation. Histamine has been found in the urine samples from IC/BPS patients [62,63]. Other inflammatory mediators are also increased. For instance, IC/BPS patients have a higher expression of inducible nitric oxide (NO) synthase mRNA in the urothelium [53,64], and in the sub-urothelial macrophages [64]. There is also a significant increase in the luminal concentration of NO [64]. NO is a signaling molecule that is believed to have an anti-inflammatory effect in the normal condition, but its over production in pathophysiological conditions can lead to it acting as a pro-inflammatory mediator [65]. Gene expression of other inflammatory mediators is also increased in patients with IC/BPS. These include genes for FGF7, CCL21 [66], IL-6, IL-10 and IL-17A [53]. At a protein level, an upregulation of urinary IL-17A [53] which is a mediator for pro-inflammatory responses [67,68], and urinary IL-6 [63], which is mainly produced in the case of tissue injury and infections [69], has been reported.

The increases in inflammatory mediators are not just limited to the bladder, as an enhanced plasma IL-6 [70] has also been detected in IC/BPS patients. Another study shows higher levels of C-reactive protein and pro-inflammatory cytokines/chemokines, *e.g.*, IL-1β, IL-6, TNF-α and IL-8 in the serum of patients with IC/BPS compared to controls [71]. An interesting study demonstrated elevated serum levels of MIG/CXCL9, IP-10/CXCL10 and I-TAC/CXCLII in IC/BPS patients. Elevated CD4+ T cells, mast cells, natural killer (NK) cells, and NKT cells have been shown at the systemic and mucosal sites in a mouse model of cyclophosphamide induced hemorrhagic cystitis [72]. Also, in a cyclophosphamide model of IC/BPS, the infiltration of inflammatory cells such as macrophages and neutrophils has been described along with an increase in TNF-α and IL-1β in the urothelial and sub-urothelial layer of the bladder [73]. All these data strongly support the role of inflammation in the pathophysiology of IC/BPS. Increased densities of inflammatory cells in the bladder have been described and increased concentrations of inflammatory mediators are believed to contribute to the etiology of the disease.

Role of sensory nerve fibers

As mentioned above, the key symptoms associated with IC/BPS are pain and urgency. Bladder sensations, such as a frequent need to void, pain and urgency, are carried on sub-urothelial afferent nerves. Since the 1990s, it has been known that the density of nerve fibers was increased in patients with IC/BPS, compared to normal controls [74]. The increase in mast cells and histamine in bladder washings has been associated with an increase in nerve fibers in both the sub-urothelial layer and in the detrusor muscle of IC/BPS patients [75]. It is also likely that the increased density of nerves fibers is related to increased levels of nerve growth factor seen in the serum [71], biopsies [36] and urine [76] of IC/BPS patients. Urinary nerve growth factor levels also correlate with symptoms of urgency [76] and it is believed that the increased nerve density may explain the symptoms of both pain and urgency.

In IC/BPS patients, a specific increase in the sensory afferent nerves located in the sub-urothelial space has been reported [36,38,76]. There is a correlation between the expression of afferent nerves that are immunoreactive for TRPV1 and the pain and urgency scores in IC/BPS patients [36] and a correlation between the density of PGP9.5-immunoreactive nerve fibers and inflammation severity in IC/BPS patients [36]. The concentration of substance P containing afferent sensory nerves is also increased in the sub-urothelial space [38] of IC/BPS patients. Substance P is a neuropeptide that plays an important role in inflammation and is overexpressed in many inflammatory conditions [77-84]. One study has suggested that an increase of sub-urothelial nerve fibers is specifically in ulcerative IC/BPS but not seen in biopsies from patients with non-ulcerative IC/BPS [75]. These studies indicate that it is likely that the enhanced pain felt by patients with IC/BPS during

bladder filling is related to the increased density of afferent nerves in the sub-urothelial layer. Increased ATP release in IC/BPS patients also seems to have a role in sensitization of afferent nerves, which will be discussed in detail in the next section.

Overall there is much evidence of inflammation in IC/BPS patients. This includes macroscopic evidence of bleeding and ulceration seen on cystoscopy. Biopsies from these patients revealed enhanced nerve density, infiltration of inflammatory cells and urothelial damage. In addition, urine and blood samples taken from IC/BPS patients have shown increased levels of pro-inflammatory mediators. The evidence for a significant role of inflammation to the pathophysiology of IC/BPS is summarized in **Figure 1**.
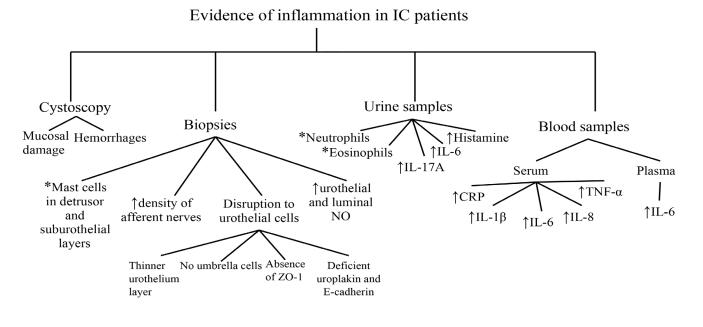
Figure 1. A summary of inflammation evidence based on the results of different studies which have been performed on IC/BPS patients and their biopsy, urine and blood samples. ↑indicates increased; *indicates infiltration.

ATP AS A SIGNALING MOLECULE FOR BLADDER PAIN AND URGENCY

In the early 1970s, Burnstock first reported the role of purinergic nerves as a non-adrenergic non-cholinergic nervous system, with ATP as its signaling molecule [85]. The purinergic system consists of two main groups of receptors; P1 and P2 receptors. P1 receptors, which are also known as adenosine receptors, have been shown to be expressed in urinary bladder where they exert an inhibitory action during the micturition reflex and inhibit cholinergic neurotransmission [86-89]. Purinergic P2 receptors are further divided into two sub-classes of P2Y and P2X. P2Y receptors, which respond preferentially to ADP and UTP, are G-protein coupled receptors and P2X receptors, responding to ATP are a group of ligand-gated ion channels [90].

The expression of purinergic receptors from both P2X and P2Y families has been shown in different layers of the urinary bladder. All seven P2X receptors have been localized in the urinary bladder of rodents [91-93], guinea pig [94] and also in human [95], using functional characterization [93], reverse transcription-polymerase chain reaction [91,96,97], immunohistochemistry [92-95] and western blot analysis [94]. In the human urinary bladder, P2X1 receptor is localized mainly on smooth muscle and to a lesser extent on mucosa [98], P2X2 receptor is present on the urothelium, smooth muscle and myofibroblast cells of the suburothelium [92,95], P2X3 receptor has been localized to the urothelium, sub-urothelial myofibroblasts and the cells surrounding smooth muscle bundles [95], as well as on suburothelial afferent nerves [99]. P2X7 receptor immunoreactivity is abundant on both smooth muscle and urothelium [95].

There are eight subtypes of the human P2Y receptors. The expression of P2Y1 [96,100], P2Y2 [96,100,101], P2Y4 [100,101], P2Y6 [102,103] and P2Y11 receptors [96] has been shown in the urothelium layer of the urinary bladder of cat [100], rat [101-103] and human [96], using RT-PCR [96], western blotting [101] and immunostaining techniques [100,102,103]. Expression of P2Y2, P2Y4 and P2Y6 have also been observed in guinea pig suburothelial myofibroblasts by immunostaining [104].

Under the normal condition, the sub-urothelial afferent nerves are activated by ATP released from the urothelium [105,106] in response to stretch of the urothelial cells [99,107-111]. Several mechanisms for ATP release have been identified, including both vesicular and non-vesicular pathways [112,113]. Vesicular ATP release is mediated by the exocytosis of ATP-enriched vesicles [112] from both neurons upon action potential [114,115] and non-excitatory cells such as urothelial cells [111].

ATP release from the urothelium is not just occurring through vesicles but also involves the activation of channels and pores in the urothelial cell membrane. One such mechanism involves pannexin channels [116]. Pannexin channels are closed under resting conditions. However they will be activated by ligand binding to membrane receptors such as ATP binding to P2X7 receptor [117]. It has been shown that pannexin-1 channel and P2X7 receptor are co-expressed in urothelial cells [118] and binding of ATP to P2X7 receptor triggers the opening of the pannexin pore [118] which causes a further release of ATP. Similarly, ATP release

from the urothelium can also be triggered by the activation of P2Y receptors [119,120]. Also, after its cellular release, ATP can be rapidly metabolized to ADP, AMP and adenosine and activate other purinergic receptors to mediate smooth muscle relaxation or contraction [121-124].

ATP released from the urothelium, in response to stretch, is an important signaling molecule that modulates the sensation of fullness felt in the bladder and mediates the voiding reflex via afferent sensory nerves [125,126]. However, an analysis of single unit pelvic afferent activities has shown that ATP is involved in both non-nociceptive (physiological) and nociceptive (painful) signal transduction [126], supporting the notion that in the urinary bladder, both high and low threshold afferent nerve fibers are sensitive to ATP. Therefore, one of the primary roles of ATP following its release from the urothelium is to activate sensory afferent nerves. The process is mediated by purinergic receptors [92,95,127]. Agonists of purinergic P2X receptors, ATP and α, β-meATP have been shown to trigger bladder afferent nerve activity which was attenuated in P2X3 knock out mice and by P2X3 antagonists [99], demonstrating that P2X3 receptors are responsible for ATP elicited responses on afferent nerves. ATP release has been shown to be increased in the bladder of both patients with IC/BPS [107,108] and animal models of IC/BPS [111]. In patients with IC/BPS, urinary levels of ATP were significantly higher than that of controls [107]. In addition, stretch induced release of ATP was enhanced in bladder strips [128] and urothelial cells from patients with IC/BPS [108]. Similar to the reports in human IC/BPS, a significant elevation in stretch-evoked ATP release has been demonstrated in a feline model of IC/BPS [111] and in cyclophosphamide-induced chronic (hemorrhagic) bladder inflammation in rats [129]. The high level of ATP and other urinary contents may diffuse through the leaky urothelium to sensitize bladder afferents, causing pain sensation and stimulating voiding, which are the typical symptoms of IC/BPS [130].

Blocking the release of ATP has shown promising results as a potential treatment for IC/BPS patients to improve the symptoms of pain, urgency and frequency. As an example, BTX-A has been used with some success in the treatment of IC/BPS [19-21]. BTX-A was shown to inhibit the stretch-mediated release of ATP from the inflamed rat bladder [129] and have anti-pain effects in IC/BPS patients [131]. Studies on the effect of BTX-A in IC/BPS has been reviewed in Chiu *et al.* [132]. BTX-A doesn't target purinergic receptors directly, but it can potentially inhibit the activation of the purinergic receptors by blocking the release of ATP.

It is well accepted that afferent nerve density and ATP release are increased in patients with IC/BPS. Therefore, targeting these pathways might influence the symptoms of pain, urgency and frequency experienced by IC/BPS patients. There have been some studies to examine these possibilities. Studies in P2X2 and P2X3 knock out mice have demonstrated marked urinary bladder hyporeflexia with increased voiding volume, reduced voiding frequency and reduced response to inflammatory pain, suggesting a prominent role of P2X2 and P2X3 receptors in mechanosensory transduction and physiological voiding reflexes [106,133].

In addition, antagonists targeting these receptors have been investigated as a potential treatment for patients with IC/BPS. P2X2/3 receptor antagonist AF-219, which is metabolically stable and has oral bioavailability, has recently been used as a therapeutic agent in IC/BPS patients with improvement in both pain and urinary urgency symptoms in treated patients [134]. In a model of cyclophosphamide-induced cystitis in rats, signs of bladder overactivity such as contraction and reduced micturition volume have been shown to be significantly improved by A-317491, a selective P2X3 receptor antagonist [135]. This antagonist has also demonstrated outstanding positive effects in a rat model of detrusor hyperreflexia, including reduced frequency of voiding and inhibition of non-micturition bladder contractions [136]. However, as mentioned previously, P2X2 and P2X3 are not the only purinergic receptors present in the urinary bladder. Other P2X receptors have also been localized within the urinary bladder [92-95].

P2X7 PURINERGIC RECEPTOR—A MODULATOR OF INFLAMMATION

The P2X7 receptor is of particular interest in IC/BPS as this receptor has a possible role in inflammation [137]. P2X7 receptors have been detected in both, urothelium and detrusor muscle cells [92-95], However, the definitive expression profile of this receptor, especially in the bladder of IC/BPS, still needs to be determined. In a mouse model of hemorrhagic cystitis induced by cyclophosphamide, Martins and associates demonstrated an upregulation of P2X7 receptors in the submucosal layer [73]. P2X7 receptors are also expressed by many immune cells including macrophages, monocytes, natural killer cells, B-lymphocytes and T-lymphocytes [138]. As described previously, many immune cells are seen to be increased in the sub-urothelial space in IC/BPS patients. A few genome profiling studies have been completed in samples from IC/BPS patients and the results demonstrate an up-regulation of genes associated with inflammatory cells mainly leukocytes. Although the changes of P2X7 receptor and other purinergic receptors have not been mentioned in these studies, it cannot be ruled out that P2X7 receptor may contribute to leukocyte infiltration in these patients [139-141].

P2X7 receptors are also known to regulate the expression, and secretion of various cytokines and other inflammatory mediators including IL-1β, IL-1 receptor antagonist (IL-1Ra), IL-2, IL-4, IL-6, IL-13, IL-18, TNF-α, NO, and superoxide anions [142]. Increases in many of these have been reported in IC/BPS patients [53,63,64,70,71]. In addition, sustained activation of P2X7 receptors is associated with apoptotic cell death [143], such as that seen in the urothelium of IC/BPS patients. Also, there have been studies implicating both ATP and P2X7 receptors in inflammasome activation which leads to the activation of the inflammatory cascade [144]. In this study, P2X7 receptors and ATP were seen to be integral to the macrophage mediated immune response against virus particles, showing the importance of the activation of P2X7 receptors in the induction of the inflammatory response [144].

Recently, many researchers have wondered if P2X7 receptor antagonists could be utilized as a treatment option for bladder diseases [73,145,146]. In 2012, Martins and associates investigated the effects of P2X7 receptor antagonists, or genetic deletion of P2X7 receptor, in cyclophosphamide-induced hemorrhagic cystitis in mice [73]. They found a decrease in the pain response in the cyclophosphamide treated animals following either pre-treatment with a P2X7 receptor antagonist or genetic removal of P2X7 receptors [73]. They also reported that the treatments led to decreased inflammatory responses and reduced edema and hemorrhage, based on macroscopic and histological assessments [73]. Also, other interesting results were observed in the models of P2X7 receptor antagonist treatment, including reduced myeloperoxidase activity and reduction in the level of IL-1β and TNF-α in bladder [73]. Myeloperoxidase is an enzyme mainly expressed in neutrophils and monocytes and plays an important role in their antimicrobial activity [147].

While the effect of P2X7 receptor antagonists in urinary bladder inflammation needs further study, there are other models of inflammation that indicate the important role of P2X7 receptors. In 2002, a selective antagonist of P2X7 receptor, oxidized ATP, was seen to have an inhibitory effect on the inflammatory pain associated with arthritis in rats [148]. The results showed that the ATP level in the inflamed tissues was reduced and the pain was relieved in oxidized ATP treated animals [148], together with a reduction in P2X7 receptor expression in both the endothelium and on peripheral nerves [148]. A study on P2X7 receptor knockout mice showed a reduction of hypersensitivity in both inflammatory and neuropathic pain models [149]. They suggested that P2X7 receptor plays a regulatory role on the production of IL-1β, which is an effective pathway in the development of pain [149].

The P2X7 receptor has also been shown to have a role in inflammatory pain in kidneys. In P2X7 receptor knockout mice there was a decrease in the number of inflammatory cells following unilateral ureteral obstruction [146]. Also, less TGF-β1, lower macrophage infiltration and reduced tubular apoptosis were observed in the P2X7 receptor knock-out animals [146]. In another study, treatment with a P2X7 receptor antagonist, A438079, resulted in reduced expression of chemokines, MCP-1 and RANTES, in a model of ischemic acute kidney injury in mice [150]. Similarly, it has been demonstrated that a reduced production of cytokines, TNF and IL-1β, occurred in the colon of colitis mice treated with the P2X7 receptor antagonist A438079 [151]. These results encourage further investigations to see if P2X7 blockage or deletion has any effect on pain associated with inflammation in IC/BPS.

CONCLUSION

The etiology of IC/BPS is still unknown although the importance of inflammation to the pathophysiology of this disorder is well supported. Increased levels of many inflammatory mediators are seen in IC/BPS patients and symptoms such as pain and urgency have been associated with the degree of inflammation present in IC/BPS patients. Recent evidence has highlighted the role of purinergic receptors, specifically the P2X7 receptor, in the process of inflammation. In terms of IC/BPS, the important role of these receptors has been demonstrated in cyclophosphamide-induced hemorrhagic cystitis and results strongly support the idea of the crucial role of P2X7 receptor in the development of inflammation. It has also been confirmed that genetic deletion of this receptor (in P2X7 receptor knock-out mice) or blocking it with an antagonist shows a significant reduction in inflammatory mediator release and a distinguished improvement in the symptoms. However, further studies are needed to reveal all the aspects of this process to be able to evaluate the treatment efficacy and clinical utility.

Author contributions

Taidi Z searched the literature and wrote and edited the manuscript. Sana-Ur-Rehman H wrote the manuscript. Bates L was the clinical consultant. Mansfield KJ and Liu L edited the manuscript.

References

1. Cox A, Golda N, Nadeau G, Curtis Nickel J, Carr L, et al. (2016) CUA guideline: Diagnosis and treatment of interstitial cystitis/bladder pain syndrome. Can Urol Assoc J 10: E136-E155. doi: 10.5489/cuaj.3786. PMID: 27790294
2. Warren JW, Meyer WA, Greenberg P, Horne L, Diggs C, et al. (2006) Using the International Continence Society's definition of painful bladder syndrome. Urology 67: 1138-1143. doi: 10.1016/j.urology.2006.01.086. PMID: 16765165
3. van de Merwe, P. (Joop) , Nordling J, Bouchelouche P, Bouchelouche K, Cervigni M, et al. (2007) Diagnostic criteria, classification, and nomenclature for painful bladder syndrome/interstitial cystitis: an ESSIC proposal. Eur Urol 53: 60-67. doi: 10.1016/j.eururo.2007.09.019. PMID: 17900797
4. Abrams P, Cardozo L, Fall M, Griffiths D, Rosier P, et al. (2002) The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. Am J Obstet Gynecol 187: 116-126. doi: 10.1067/mob.2002.125704. PMID: 12114899
5. Horn T, Holm NR, Hald T (1998) Interstitial cystitis. Ultrastructural observations on detrusor smooth muscle cells. APMIS 106: 909-916. PMID: 9808418
6. Bogart LM, Berry SH, Clemens JQ (2007) Symptoms of interstitial cystitis, painful bladder syndrome and similar diseases in women: a systematic review. J Urol 177: 450-456. doi: 10.1016/j.juro.2006.09.032. PMID: 17222607
7. Foster HE Jr, Hanno PM, Nickel JC, Payne CK, Mayer RD, et al. (2010) Effect of amitriptyline on symptoms in treatment naive patients with interstitial cystitis/painful bladder syndrome. J Urol 183: 1853-18588. doi: 10.1016/j.juro.2009.12.106. PMID: 20303115
8. Han E, Nguyen L, Sirls L, Peters K (2018) Current best practice management of interstitial cystitis/bladder pain syndrome. Ther Adv Urol 10: 197-211. doi: 10.1177/1756287218761574. PMID: 30034539
9. van Ophoven A, Pokupic S, Heinecke A, Hertle L (2004) A prospective, randomized, placebo controlled, double-blind study of amitriptyline for the treatment of interstitial cystitis. J Urol 172: 533-536. doi: 10.1097/01.ju.0000132388.54703.4d. PMID: 15247722
10. Hanno PM, Burks DA, Clemens JQ, Dmochowski RR, Erickson D, et al. (2011) AUA guideline for the diagnosis and treatment of interstitial cystitis/bladder pain syndrome. J Urol 185: 2162-2170. doi: 10.1016/j.juro.2011.03.064. PMID: 21497847
11. Sairanen J, Tammela TL, Leppilahti M, Multanen M, Paananen I, et al. (2005) Cyclosporine A and pentosan polysulfate sodium for the treatment of interstitial cystitis: a randomized comparative study. J Urol 174: 2235-2238. doi: 10.1097/01.ju.0000181808.45786.84. PMID: 16280777
12. Forrest JB, Payne CK, Erickson DR (2012) Cyclosporine A for refractory interstitial cystitis/bladder pain syndrome: experience of 3 tertiary centers. J Urol 188: 1186-1191. doi: 10.1016/j.juro.2012.06.023. PMID: 22901569
13. Theoharides TC, Sant GR (1997) Hydroxyzine therapy for interstitial cystitis. Urology 49: 108-110. PMID: 9146011
14. Perez-Marrero R, Emerson LE, Feltis JT (1988) A controlled study of dimethyl sulfoxide in interstitial cystitis. J Urol 140: 36-39. PMID: 3288775
15. Parsons CL, Housley T, Schmidt JD, Lebow D (1994) Treatment of interstitial cystitis with intravesical heparin. Br J Urol 73: 504-507. doi: 10.1111/j.1464-410X.1994.tb07634.x. PMID: 8012771
16. Parsons CL, Zupkas P, Proctor J, Koziol J, Franklin A, et al. (2012) Alkalinized lidocaine and heparin provide immediate relief of pain and urgency in patients with interstitial cystitis. J Sex Med 9: 207-212. doi: 10.1111/j.1743-6109.2011.02542.x. PMID: 22082303
17. Stav K, Beberashvili I, Lindner A, Leibovici D (2012) Predictors of response to intravesical dimethyl-sulfoxide cocktail in patients with interstitial cystitis. Urology 80: 61-65. doi: 10.1016/j.urology.2012.03.030. PMID: 22748865
18. Kuo H (2013) Repeated onabotulinumtoxin-a injections provide better results than single injection in treatment of painful bladder syndrome. Pain Physician 16: E15-23. PMID: 23340540
19. Giannantoni A, Costantini E, Di Stasi SM, Tascini MC, Bini V, et al. (2006) Botulinum A toxin intravesical injections in the treatment of painful bladder syndrome: a pilot study. Eur Urol 49: 704-709. doi: 10.1016/j.eururo.2005.12.002. PMID: 16417964
20. Giannantoni A, Mearini E, Del Zingaro M, Proietti S, Porena M (2010) Two-year efficacy and safety of botulinum a toxin intravesical injections in patients affected by refractory painful bladder syndrome. Curr Drug Deliv 7: 1-4. PMID: 19863481
21. Pinto R, Lopes T, Frias B, Silva A, Silva JA, et al. (2010) Trigonal injection of botulinum toxin A in patients with refractory bladder pain syndrome/interstitial cystitis. Eur Urol 58: 360-365. doi: 10.1016/j.eururo.2010.02.031. PMID: 20227820
22. Barua JM, Arance I, Angulo JC, Riedl CR (2016) A systematic review and meta-analysis on the efficacy of intravesical therapy for bladder pain syndrome/interstitial cystitis. Int Urogynecol J 27: 1137-1147. doi: 10.1007/s00192-015-2890-7. PMID: 26590137
23. Sant GR, Propert KJ, Hanno PM, Burks D, Culkin D, et al. (2003) A pilot clinical trial of oral pentosan polysulfate and oral hydroxyzine in patients with interstitial cystitis. J Urol 170: 810-815. doi: 10.1097/01.ju.0000083020.06212.3d.

PMID: 12913705

24. Bade JJ, Laseur M, Nieuwenburg A, van der Weele LT, Mensink HJ (1997) A placebo-controlled study of intravesical pentosanpolysulphate for the treatment of interstitial cystitis. Br J Urol 79: 168-171. doi: 10.1046/j.1464-410X.1997.03384.x. PMID: 9052464

25. Sairanen J, Leppilahti M, Tammela TL, Paananen I, Aaltomaa S, et al. (2009) Evaluation of health-related quality of life in patients with painful bladder syndrome/interstitial cystitis and the impact of four treatments on it. Scand J Urol Nephrol 43: 212-219. doi: 10.1080/00365590802671031. PMID: 19137459

26. Tutolo M, Ammirati E, Castagna G, Klockaerts K, Plancke H, et al. (2017) A prospective randomized controlled multicentre trial comparing intravesical DMSO and chondroitin sulphate 2% for painful bladder syndrome/interstitial cystitis. Int Braz J Urol 43:134-141. Epub PubMed Central PMCID: 134-141. doi: 10.1590/s1677-5538.Ibju.2016.0302. PMID: 28124536

27. Johansson SL, Fall M (1990) Clinical features and spectrum of light microscopic changes in interstitial cystitis. J Urol 143: 1118-1124. PMID: 2342171

28. Logadottir Y, Fall M, Kåbjörn-Gustafsson C, Peeker R (2012) Clinical characteristics differ considerably between phenotypes of bladder pain syndrome/interstitial cystitis. Scand J Urol Nephrol 46: 365-370. doi: 10.3109/00365599.2012.689008. PMID: 22607036

29. Tomoe H (2015) In what type of interstitial cystitis/bladder pain syndrome is DMSO intravesical instillation therapy effective?. Transl Androl Urol 4: 600-604. doi: 10.3978/j.issn.2223-4683.2015.09.01. PMID: 26816859

30. Lee JE, Yi BH, Lee HK, Lee MH, Kim YH (2015) Correlation of cystoscopically confirmed periureterally located hunner lesion with vesicoureteral reflux: preliminary study in patients with interstitial cystitis. AJR Am J Roentgenol 204: W457-W460. doi: 10.2214/ajr.14.13108. PMID: 25794095

31. Grover S, Srivastava A, Lee R, Tewari AK, Te AE (2011) Role of inflammation in bladder function and interstitial cystitis. Ther Adv Urol 3: 19-33. doi: 10.1177/1756287211398255. PMID: 21789096

32. Liu HT, Jiang YH, Kuo HC (2015) Alteration of urothelial inflammation, apoptosis, and junction protein in patients with various bladder conditions and storage bladder symptoms suggest common pathway involved in underlying pathophysiology. Low Urin Tract Symptoms 7: 102-107. doi: 10.1111/luts.12062. PMID: 26663690

33. Eldrup J, Thorup J, Nielsen SL, Hald T, Hainau B (1983) Permeability and ultrastructure of human bladder epithelium. Br J Urol 55: 488-492. PMID: 6626894

34. Said JW, Van de Velde R, Gillespie L (1989) Immunopathology of interstitial cystitis. Mod Pathol 2: 593-602. PMID: 2685811

35. Parsons CL, Lilly JD, Stein P (1991) Epithelial dysfunction in nonbacterial cystitis (interstitial cystitis). J Urol 145: 732-735. PMID: 2005689

36. Liu BL, Yang F, Zhan HL, Feng ZY, Zhang ZG, et al. (2014) Increased severity of inflammation correlates with elevated expression of TRPV1 nerve fibers and nerve growth factor on interstitial cystitis/bladder pain syndrome. Urol Int 92: 202-208. doi: 10.1159/000355175. PMID: 24458144

37. Pang X, Cotreau-Bibbo MM, Sant GR, Theoharides TC (1995) Bladder mast cell expression of high affinity oestrogen receptors in patients with interstitial cystitis. Br J Urol 75: 154-161. PMID: 7850318

38. Pang X, Marchand J, Sant GR, Kream RM, Theoharides TC (1995) Increased number of substance P positive nerve fibres in interstitial cystitis. Br J Urol 75: 744-750. doi: 10.1111/j.1464-410X.1995.tb07384.x. PMID: 7542136

39. Yamada T, Murayama T, Mita H, Akiyama K (2000) Subtypes of bladder mast cells in interstitial cystitis. Int J Urol 7: 292-297. doi: 10.1046/j.1442-2042.2000.00197.x. PMID: 10976817

40. Slobodov G, Feloney M, Gran C, Kyker KD, Hurst RE, et al. (2004) Abnormal expression of molecular markers for bladder impermeability and differentiation in the urothelium of patients with interstitial cystitis. J Urol 171: 1554-1558. doi: 10.1097/01.ju.0000118938.09119.a5. PMID: 15017219

41. Graham E, Chai TC (2006) Dysfunction of bladder urothelium and bladder urothelial cells in interstitial cystitis. Curr Urol Rep 7: 440-446. doi: 10.1007/s11934-006-0051-8. PMID: 17052438

42. Montalbetti N, Rued AC, Clayton DR, Ruiz WG, Bastacky SI, et al. (2015) Increased urothelial paracellular transport promotes cystitis. Am J Physiol Renal Physiol 309: F1070-F1081. Epub PubMed Central PMCID: doi: 10.1152/ajprenal.00200.2015. PMID: 26423859

43. Sasisekharan R, Raman R, Prabhakar V (2006) Glycomics approach to structure-function relationships of glycosaminoglycans. Annu Rev Biomed Eng 8: 181-231. doi: 10.1146/annurev.bioeng.8.061505.095745. PMID: 16834555

44. Hurst RE, Roy JB, Min KW, Veltri RW, Marley G, et al. (1996) A deficit of chondroitin sulfate proteoglycans on the bladder uroepithelium in interstitial cystitis. Urology 48: 817-821. doi: 10.1016/s0090-4295(96)00322-6. PMID: 8911536

45. Hurst RE (2003) A deficit of proteoglycans on the bladder uroepithelium in interstitial cystitis. Eur Urol Suppl 2: 10-13. doi: 10.1016/S1569-9056(03)00033-2.

46. Lavelle JP, Negrete HO, Poland PA, Kinlough CL, Meyers SD, et al. (1997) Low permeabilities of MDCK cell monolayers: a model barrier epithelium. Am J Physiol 273: doi: 10.1152/ajprenal.1997.273.1.F67. PMID: 9249593

47. Huygens A, Crnolatac I, Maes J, Cleynenbreugel B, Van Poppel H, et al. (2007) Influence of the glycosaminoglycan layer on the permeation of hypericin in rat bladders in vivo. BJU international 100: 1176-1181. doi: 10.1111/j.1464-410X.2007.07167.x. PMID: 17868424

48. Lewis SA (2000) Everything you wanted to know about the bladder epithelium but were afraid to ask. Am J Physiol Renal Physiol 278: doi: 10.1152/ajprenal.2000.278.6.F867. PMID: 10836974

49. Lewis SA, Berg JR, Kleine TJ (1995) Modulation of epithelial permeability by extracellular macromolecules. Physiol Rev 75: 561-589. doi: 10.1152/physrev.1995.75.3.561. PMID: 7624394

50. Cvach K, Rosamilia A (2015) Review of intravesical therapies for bladder pain syndrome/interstitial cystitis. Transl Androl Urol 4: 629-637. doi: 10.3978/j.issn.2223-4683.2015.10.07. PMID: 26816864

51. Katnik-Prastowska I, Lis J, Matejuk A (2014) Glycosylation of uroplakins. Implications for bladder physiopathology. Glycoconj J 31: 623-636. doi: 10.1007/s10719-014-9564-4. PMID: 25394961

52. Aldenborg F, Fall M, Enerbäck L (1986) Proliferation and transepithelial migration of mucosal mast cells in interstitial cystitis. Immunology 58: 411-416. PMID: 3733146

53. Logadottir Y, Delbro D, Fall M, Gjertsson I, Jirholt P, et al. (2014) Cytokine expression in patients with bladder pain syndrome/interstitial cystitis ESSIC type 3C. J Urol 192: 1564-1568. doi: 10.1016/j.juro.2014.04.099. PMID: 24813342

54. Dodd LG, Tello J (1998) Cytologic examination of urine from patients with interstitial cystitis. Acta Cytol 42: 923-927. doi: 10.1159/000331969. PMID: 9684578

55. Kolaczkowska E, Kubes P (2013) Neutrophil recruitment and function in health and inflammation. Nat Rev Immunol 13: 159-175. doi: 10.1038/nri3399. PMID: 23435331

56. Fulkerson PC, Rothenberg ME (2013) Targeting eosinophils in allergy, inflammation and beyond. Nat Rev Drug Discov 12: 117-129. doi: 10.1038/nrd3838. PMID: 23334207

57. Fall M, Johansson SL, Aldenborg F (1987) Chronic interstitial cystitis: a heterogeneous syndrome. J Urol 137: 35-38. PMID: 3795363

58. Zhong CJ, Wang K, Zhang L, Yang CQ, Zhang K, et al. (2015) Mast cell activation is involved in stress-induced epithelial barrier dysfunction in the esophagus. J Dig Dis 16: 186-196. doi: 10.1111/1751-2980.12226. PMID: 25565566

59. Peeker R, Enerback L, Fall M, Recruitment AF (2000) Recruitment, distribution and phenotypes of mast cells in interstitial cystitis. J Urol 163: 1009-1015. PMID: 10688040

60. Hofmeister MA, He F, Ratliff TL, Mahoney T, Becich MJ (1997) Mast cells and nerve fibers in interstitial cystitis (IC): An algorithm for histologic diagnosis via quantitative image analysis and morphometry (QIAM). Urology 49: 41-47. doi: 10.1016/S0090-4295(99)80330-6. PMID: 9146000

61. Sant GR, Theoharides TC (1994) The role of the mast cell in interstitial cystitis. Urol Clin North Am 21: 41-53. PMID: 8284844

62. Holm-Bentzen M, Søndergaard I, Hald T (1987) Urinary excretion of a metabolite of histamine (1,4-methyl-imidazole-acetic-acid) in painful bladder disease. Br J Urol 59: 230-233. PMID: 3567484

63. Lamale LM, Lutgendorf SK, Zimmerman MB, Kreder KJ (2006) Interleukin-6, histamine, and methylhistamine as diagnostic markers for interstitial cystitis. Urology 68: 702-706. doi: 10.1016/j.urology.2006.04.033. PMID: 17070335

64. Koskela LR, Thiel T, Ehren I, De Verdier PJ, Wiklund NP (2008) Localization and expression of inducible nitric oxide synthase in biopsies from patients with interstitial cystitis. J Urol 180: 737-741. doi: 10.1016/j.juro.2008.03.184. PMID: 18554637

65. Sharma JN, Al-Omran A, Parvathy SS (2007) Role of nitric oxide in inflammatory diseases. Inflammopharmacology 15: 252-259. doi: 10.1007/s10787-007-0013-x. PMID: 18236016

66. Offiah I, Didangelos A, Dawes J, Cartwright R, Khullar V, et al. (2016) The expression of inflammatory mediators in bladder pain syndrome. Eur Urol 70: 283-290. doi: 10.1016/j.eururo.2016.02.058. PMID: 26965559

67. Jin W, Dong C (2013) IL-17 cytokines in immunity and inflammation. Emerg Microbes Infect 2: e60. doi: 10.1038/emi.2013.58.

68. Ishigame H, Kakuta S, Nagai T, Kadoki M, Nambu A, et al. (2009) Differential roles of interleukin-17A and -17F in host defense against mucoepithelial bacterial infection and allergic responses. Immunity 30: 108-19. doi: 10.1016/j.immuni.2008.11.009. PMID: 19144317

69. Tanaka T, Narazaki M, Kishimoto T (2014) IL-6 in inflammation, immunity, and

disease. Cold Spring Harb Perspect Biol 6: a016295. doi: 10.1101/cshperspect.a016295. PMID: 25190079

70. Schrepf A, O'Donnell M, Luo Y, Bradley CS, Kreder K, et al. (2014) Inflammation and inflammatory control in interstitial cystitis/bladder pain syndrome: Associations with painful symptoms. Pain 155: 1755-1761. doi: 10.1016/j.pain.2014.05.029. PMID: 24907404

71. Jiang YH, Peng CH, Liu HT, Kuo HC (2013) Increased pro-inflammatory cytokines, C-reactive protein and nerve growth factor expressions in serum of patients with interstitial cystitis/bladder pain syndrome. PLoS One 8: e76779. doi: 10.1371/journal.pone.0076779. PMID: 24146927

72. Sakthivel SK, Singh UP, Singh S, Taub DD, Novakovic KR, et al. (2008) CXCL10 blockade protects mice from cyclophosphamide-induced cystitis. J Immune Based Ther Vaccines 6. doi: 10.1186/1476-8518-6-6. PMID: 18957084

73. Martins JP, Silva RB, Coutinho-Silva R, Takiya CM, Battastini AM, et al. (2011) The role of P2X7 purinergic receptors in inflammatory and nociceptive changes accompanying cyclophosphamide-induced haemorrhagic cystitis in mice. Br J Pharmacol 165: 183-196. doi: 10.1111/j.1476-5381.2011.01535.x. PMID: 21675966

74. Christmas TJ, Rode J, Chapple CR, Milroy EJ, Turner-Warwick RT (1990) Nerve fibre proliferation in interstitial cystitis. Virchows Arch A Pathol Anat Histopathol 416: 447-451. doi: 10.1007/BF01605152. PMID: 2107633

75. Lundeberg T, Liedberg H, Nordling L, Theodorsson E, Owzarski A, et al. (1993) Interstitial cystitis: correlation with nerve fibres, mast cells and histamine. Br J Urol 71: 427-429. doi: 10.1111/j.1464-410X.1993.tb15986.x. PMID: 8499987

76. Kim SW, Im YJ, Choi HC, Kang HJ, Kim JY, et al. (2014) Urinary nerve growth factor correlates with the severity of urgency and pain. Int Urogynecol J 25: 1561-1567. doi: 10.1007/s00192-014-2424-8. PMID: 24866276

77. Yaraee R, Ebtekar M, Ahmadiani A, Sabahi F (2003) Neuropeptides (SP and CGRP) augment pro-inflammatory cytokine production in HSV-infected macrophages. Int Immunopharmacol 3: 1883-1887. doi: 10.1016/s1567-5769(03)00201-7. PMID: 14636837

78. Azzolina A, Bongiovanni A, Lampiasi N (2003) Substance P induces TNF-alpha and IL-6 production through NF kappa B in peritoneal mast cells. Biochim Biophys Acta 1643: 75-83. PMID: 14654230

79. Ang SF, Moochhala SM, MacAry PA, Bhatia M (2011) Hydrogen sulfide and neurogenic inflammation in polymicrobial sepsis: involvement of substance P and ERK-NF-kappaB signaling. PLoS One 6: e24535. doi: 10.1371/journal.pone.0024535. PMID: 21931742

80. Bhatia M, Zhi L, Zhang H, Ng S, Moore PK (2006) Role of substance P in hydrogen sulfide-induced pulmonary inflammation in mice. Am J Physiol Lung Cell Mol Physiol 291: doi: 10.1152/ajplung.00053.2006. PMID: 16798781

81. Hegde A, Tamizhselvi R, Manikandan J, Melendez AJ, Moochhala SM, et al. (2010) Substance P in polymicrobial sepsis: molecular fingerprint of lung injury in preprotachykinin-A-/- mice. Mol Med 16: 188-198. doi: 10.2119/molmed.2009.00166. PMID: 20454520

82. Sio SWS, Ang SF, Lu J, Moochhala S, Bhatia M (2010) Substance P upregulates cyclooxygenase-2 and prostaglandin E metabolite by activating ERK1/2 and NF-kappaB in a mouse model of burn-induced remote acute lung injury. J Immunol 185: 6265-6276. doi: 10.4049/jimmunol.1001739. PMID: 20926798

83. Sio SW, Puthia MK, Lu J, Moochhala S, Bhatia M (2008) The neuropeptide substance P is a critical mediator of burn-induced acute lung injury. J immunol 180: 8333-8341. PMID: 18523300

84. Sipos G, Sipos P, Altdorfer K, Pongor E, Feher E (2008) Correlation and immunolocalization of substance P nerve fibers and activated immune cells in human chronic gastritis. Anat Rec (Hoboken) 291: 1140-1148. doi: 10.1002/ar.20737. PMID: 18727057

85. Burnstock G (1972) Purinergic nerves. Pharmacol Rev 24: 509-581. PMID: 4404211

86. Kitta T, Chancellor MB, de Groat WC, Kuno S, Nonomura K, et al. (2013) Roles of adenosine A1 and A2A receptors in the control of micturition in rats. Neurourol Urodyn 33: 1259-1265. doi: 10.1002/nau.22487. PMID: 24014261

87. Silva I, Costa AF, Moreira S, Ferreirinha F, Magalhaes-Cardoso MT, et al. (2017) Inhibition of cholinergic neurotransmission by beta3-adrenoceptors depends on adenosine release and A1-receptor activation in human and rat urinary bladders. Am J Physiol Renal Physiol 313: F388-F403. doi: 10.1152/ajprenal.00392.2016. PMID: 28446460

88. Pakzad M, Ikeda Y, McCarthy C, Kitney DG, Jabr RI, et al. (2016) Contractile effects and receptor analysis of adenosine-receptors in human detrusor muscle from stable and neuropathic bladders. Naunyn Schmiedebergs Arch Pharmacol 389: 921-929. doi: 10.1007/s00210-016-1255-1. PMID: 27185496

89. Searl TJ, Dynda DI, Alanee SR, El-Zawahry AM, McVary KT, et al. (2015) A1 Adenosine receptor-mediated inhibition of parasympathetic neuromuscular transmission in human and murine urinary bladder. J Pharmacol Exp Ther 356: 116-122. doi: 10.1124/jpet.115.228882. PMID: 26534943

90. Burnstock G (2007) Purine and pyrimidine receptors. Cell Mol Life Sci 64: 1471-1483. doi: 10.1007/s00018-007-6497-0. PMID: 17375261

91. Chen X, Gebhart GF (2010) Differential purinergic signaling in bladder sensory neurons of naïve and bladder-inflamed mice. Pain 148: 462-472. doi: 10.1016/j.pain.2009.12.006. PMID: 20045252

92. Lee HY, Bardini M, Burnstock G (2000) Distribution of P2X receptors in the urinary bladder and the ureter of the rat. J Urol 163: 2002-2007. PMID: 10799247

93. Vial C, Evans RJ (2000) P2X receptor expression in mouse urinary bladder and the requirement of P2X(1) receptors for functional P2X receptor responses in the mouse urinary bladder smooth muscle. Br J Pharmacol 131: 1489-1495. doi: 10.1038/sj.bjp.0703720. PMID: 11090125

94. Menzies J, Paul A, Kennedy C (2003) P2X7 subunit-like immunoreactivity in the nucleus of visceral smooth muscle cells of the guinea pig. Auton Neurosci 106: 103-109. doi: 10.1016/S1566-0702(03)00078-X. PMID: 12878079

95. Svennersten K, Hallen-Grufman K, de Verdier PJ, Wiklund NP, Poljakovic M (2015) Localization of P2X receptor subtypes 2, 3 and 7 in human urinary bladder. BMC Urol 15: 81. doi: 10.1186/s12894-015-0075-9. PMID: 26253104

96. Shabir S, Cross W, Kirkwood LA, Pearson JF, Appleby PA, et al. (2013) Functional expression of purinergic P2 receptors and transient receptor potential channels by the human urothelium. Am J Physiol Renal Physiol 305: F396-460. doi: 10.1152/ajprenal.00127.2013. PMID: 23720349

97. Tempest HV, Dixon AK, Turner WH, Elneil S, Sellers LA, et al. (2004) P2X and P2X receptor expression in human bladder urothelium and changes in interstitial cystitis. BJU Int 93: 1344-1348. doi: 10.1111/j.1464-410X.2004.04858.x. PMID: 15180635

98. Chua WC, Liu L, Mansfield KJ, Vaux KJ, Moore KH, et al. (2007) Age-related changes of P2X(1) receptor mRNA in the bladder detrusor from men with and without bladder outlet obstruction. Exp Gerontol 42: 686-692. doi: 10.1016/j.exger.2007.02.003. PMID: 17399929

99. Vlaskovska M, Kasakov L, Rong W, Bodin P, Bardini M, et al. (2001) P2X3 knock-out mice reveal a major sensory role for urothelially released ATP. J Neurosci 21: 5670-5677. doi: 10.1523/JNEUROSCI.21-15-05670.2001. PMID: 11466438

100. Birder LA, Ruan HZ, Chopra B, Xiang Z, Barrick S, et al. (2004) Alterations in P2X and P2Y purinergic receptor expression in urinary bladder from normal cats and cats with interstitial cystitis. Am J Physiol Renal Physiol 287: F1084-1091. doi: 10.1152/ajprenal.00118.2004. PMID: 15251862

101. Chopra B, Gever J, Barrick SR, Hanna-Mitchell AT, Beckel JM, et al. (2008) Expression and function of rat urothelial P2Y receptors. Am J Physiol Renal Physiol 294: F821-829. doi: 10.1152/ajprenal.00321.2006. PMID: 18216148

102. Carneiro I, Timoteo MA, Silva I, Vieira C, Baldaia C, et al. (2014) Activation of P2Y6 receptors increases the voiding frequency in anaesthetized rats by releasing ATP from the bladder urothelium. Br J Pharmacol 171: 3404-3419. doi: 10.1111/bph.12711. PMID: 24697602

103. Timoteo MA, Carneiro I, Silva I, Noronha-Matos JB, Ferreirinha F, et al. (2014) ATP released via pannexin-1 hemichannels mediates bladder overactivity triggered by urothelial P2Y6 receptors. Biochem Pharmacol 87: 371-379. Epub 2013/11/26. doi: 10.1016/j.bcp.2013.11.007 87: 371-9. doi: 10.1016/j.bcp.2013.11.007. PMID: 24269631

104. Sui GP, Wu C, Fry CH (2006) Characterization of the purinergic receptor subtype on guinea-pig suburothelial myofibroblasts. BJU Int 97: 1327-1331. doi: 10.1111/j.1464-410X.2006.06200.x. PMID: 16686733

105. Collins VM, Daly DM, Liaskos M, McKay NG, Sellers D, et al. (2013) OnabotulinumtoxinA significantly attenuates bladder afferent nerve firing and inhibits ATP release from the urothelium. BJU Int 112: 1018-1026. doi: 10.1111/bju.12266. PMID: 23937318

106. Cockayne DA, Hamilton SG, Zhu QM, Dunn PM, Zhong Y, et al. (2000) Urinary bladder hyporeflexia and reduced pain-related behaviour in P2X3-deficient mice. Nature 407: 1011-1015. doi: 10.1038/35039519. PMID: 11069181

107. Sun Y, Keay S, De Deyne PG, Chai TC (2001) Augmented stretch activated adenosine triphosphate release from bladder uroepithelial cells in patients with interstitial cystitis. J Urol 166: 1951-1956. PMID: 11586266

108. Sun Y, Chai TC (2005) Augmented extracellular ATP signaling in bladder urothelial cells from patients with interstitial cystitis. Am J Physiol Cell Physiol 290: C27-34. doi: 10.1152/ajpcell.00552.2004. PMID: 16107499

109. Ferguson DR, Kennedy I, Burton TJ (1998) ATP is released from rabbit urinary bladder epithelial cells by hydrostatic pressure changes--a possible sensory mechanism?. J Physiol 505: 503-511. Epub PubMed Central PMCID: PMID: 9423189

110. Sadananda P, Shang F, Liu L, Mansfield KJ, Burcher E (2009) Release of ATP from rat urinary bladder mucosa: role of acid, vanilloids and stretch. Br J Pharmacol 158: 1655-1662. doi: 10.1111/j.1476-5381.2009.00431.x. PMID: 19860742

111. Birder LA, Barrick SR, Roppolo JR, Kanai AJ, de Groat WC, et al. (2003) Feline interstitial cystitis results in mechanical hypersensitivity and altered ATP release from bladder urothelium. Am J Physiol Renal Physiol 285: F423-429. doi: 10.1152/ajprenal.00056.2003. PMID: 12759226

112. Lazarowski ER (2012) Vesicular and conductive mechanisms of nucleotide release. Purinergic Signal 8: 359-373. doi: 10.1007/s11302-012-9304-9. PMID: 22528679

113. Lohman AW, Billaud M, Isakson BE (2012) Mechanisms of ATP release and signalling in the blood vessel wall. Cardiovasc Res 2012 95: 269-280. doi: 10.1093/cvr/cvs187. PMID: 22678409

114. Burnstock G (2006) Historical review: ATP as a neurotransmitter. Trends Pharmacol Sci 27: 166-176. doi: 10.1016/j.tips.2006.01.005. PMID: 16487603

115. Ho T, Jobling AI, Greferath U, Chuang T, Ramesh A, et al. (2015) Vesicular expression and release of ATP from dopaminergic neurons of the mouse retina and midbrain. Front Cell Neurosci 9: 389. doi: 10.3389/fncel.2015.00389. PMID: 26500494

116. Sana-Ur-Rehman H, Markus I, Moore KH, Mansfield KJ, Liu L (2017) Expression and localization of pannexin-1 and CALHM1 in porcine bladder and their involvement in modulating ATP release. Am J Physiol Regul Integr Comp Physiol 312: R763-R772. doi: 10.1152/ajpregu.00039.2016. PMID: 28254749

117. Locovei S, Scemes E, Qiu F, Spray DC, Dahl G (2007) Pannexin1 is part of the pore forming unit of the P2X(7) receptor death complex. FEBS letters 581: 483-488. doi: 10.1016/j.febslet.2006.12.056. PMID: 17240370

118. Negoro H, Urban-Maldonado M, Liou LS, Spray DC, Thi MM, et al. (2014) Pannexin 1 channels play essential roles in urothelial mechanotransduction and intercellular signaling. PLoS One 9: e106269. doi: 10.1371/journal.pone.0106269. PMID: 25170954

119. Sui G, Fry CH, Montgomery B, Roberts M, Wu R, et al. (2013) Purinergic and muscarinic modulation of ATP release from the urothelium and its paracrine actions. Am J Physiol Renal Physiol 306: F286-298. doi: 10.1152/ajprenal.00291.2013. PMID: 24285497

120. Mansfield KJ, Hughes JR (2014) P2Y receptor modulation of ATP release in the urothelium. Biomed Res Int 2014: 830374-8. doi: 10.1155/2014/830374. PMID: 24829920

121. Ralevic V, Burnstock G (1998) Receptors for purines and pyrimidines. Pharmacol Rev 50: 413-492. PMID: 9755289

122. Yegutkin GG (2008) Nucleotide- and nucleoside-converting ectoenzymes: Important modulators of purinergic signalling cascade. Biochim Biophys Acta 1783: 673-694. doi: 10.1016/j.bbamcr.2008.01.024. PMID: 18302942

123. McMurray G, Dass N, Brading AF (1998) Purinoceptor subtypes mediating contraction and relaxation of marmoset urinary bladder smooth muscle. Br J Pharmacol 123: 1579-1586. doi: 10.1038/sj.bjp.0701774. PMID: 9605564

124. Fry CH, Young JS, Jabr RI, McCarthy C, Ikeda Y, et al. (2012) Modulation of spontaneous activity in the overactive bladder: the role of P2Y agonists. Am J Physiol Renal Physiol 302: F1447-1454. doi: 10.1152/ajprenal.00436.2011. PMID: 22357922

125. Birder L, Andersson KE (2013) Urothelial signaling. Physiol Rev 93: 653-680. doi: 10.1152/physrev.00030.2012. PMID: 23589830

126. Rong W, Spyer KM, Burnstock G (2002) Activation and sensitisation of low and high threshold afferent fibres mediated by P2X receptors in the mouse urinary bladder. J Physiol 541(Pt 2): 591-600. PMID: 12042363

127. Zhong Y, Banning AS, Cockayne DA, Ford APDW, Burnstock G, et al. (2003) Bladder and cutaneous sensory neurons of the rat express different functional p2x receptors. Neuroscience 120: 667-675. doi: 10.1016/S0306-4522(03)00243-4. PMID: 12895508

128. Kumar V, Chapple CR, Surprenant AM, Chess-Williams R (2007) Enhanced adenosine triphosphate release from the urothelium of patients with painful bladder syndrome: a possible pathophysiological explanation. J Urol 178: 1533-1536. doi: 10.1016/j.juro.2007.05.116. PMID: 17707056

129. Smith CP, Vemulakonda VM, Kiss S, Boone TB, Somogyi GT (2005) Enhanced ATP release from rat bladder urothelium during chronic bladder inflammation: effect of botulinum toxin A. Neurochem Int 47: 291-297. doi: 10.1016/j.neuint.2005.04.021. 47: 291-297. doi: 10.1016/j.neuint.2005.04.021. PMID: 15970360

130. Montalbetti N, Rued AC, Taiclet SN, Birder LA, Kullmann FA, et al. (2017) Urothelial tight junction barrier dysfunction sensitizes bladder afferents. eNeuro 4. doi: 10.1523/eneuro.0381-16.2017. PMID: 28560313

131. Liu H, Kuo H (2007) Intravesical botulinum toxin A injections plus hydrodistension can reduce nerve growth factor production and control bladder pain in interstitial cystitis. Urology 70: 463-468. doi: 10.1016/j.urology.2007.04.038. PMID: 17905097

132. Chiu B, Tai H, Chung S, Birder LA (2016) Botulinum Toxin A for Bladder Pain Syndrome/Interstitial Cystitis. Toxins (Basel) 8: 201. doi: 10.3390/toxins8070201. PMID: 27376330

133. Cockayne DA, Dunn PM, Zhong Y, Rong W, Hamilton SG, et al. (2005) P2X2 knockout mice and P2X2/P2X3 double knockout mice reveal a role for the P2X2 receptor subunit in mediating multiple sensory effects of ATP. J Physiol 567(Pt 2): 621-639. doi: 10.1113/jphysiol.2005.088435. PMID: 15961431

134. Ford AP, Undem BJ (2013) The therapeutic promise of ATP antagonism at P2X3 receptors in respiratory and urological disorders. Front Cell Neurosci 7: 267. doi: 10.3389/fncel.2013.00267. PMID: 24391544

135. Ito K, Iwami A, Katsura H, Ikeda M (2007) Therapeutic effects of the putative P2X3/P2X2/3 antagonist A-317491 on cyclophosphamide-induced cystitis in rats. Naunyn Schmiedebergs Arch Pharmacol 377: 483-490. doi: 10.1007/s00210-007-0197-z. PMID: 17917716

136. Lu SH, Groat WC, Lin AT, Chen KK, Chang LS (2007) Evaluation of purinergic mechanism for the treatment of voiding dysfunction: a study in conscious spinal cord-injured rats. J Chin Med Assoc 70: 439-444. PMID: 17962144

137. De Marchi E, Orioli E, Dal Ben D, Adinolfi E (2016) P2X7 Receptor as a Therapeutic Target. Adv Protein Chem Struct Biol 104: 39-79. doi: 10.1016/bs.apcsb.2015.11.004. PMID: 27038372

138. Gu BJ, Zhang WY, Bendall LJ, Chessell IP, Buell GN, et al. (2000) Expression of P2X(7) purinoceptors on human lymphocytes and monocytes: evidence for nonfunctional P2X(7) receptors. Am J Physiol Cell Physiol 279: C1189-1197. PMID: 11003599

139. Keay S, Seillier-Moiseiwitsch F, Zhang C, Chai TC, Zhang J (2003) Changes in human bladder epithelial cell gene expression associated with interstitial cystitis or antiproliferative factor treatment. Physiol Genomics 14: 107-115. doi: 10.1152/physiolgenomics.00055.2003. PMID: 12847144

140. Tseng LH, Chen I, Wang CN, Lin YH, Lloyd LK, et al. (2010) Genome-based expression profiling study of Hunner's ulcer type interstitial cystitis: an array of 40-gene model. Int Urogynecol J 21: 911-918. doi: 10.1007/s00192-010-1129-x. PMID: 20204322

141. Gamper M, Viereck V, Geissbuhler V, Eberhard J, Binder J, et al. (2009) Gene expression profile of bladder tissue of patients with ulcerative interstitial cystitis. BMC Genomics 10: 199. doi: 10.1186/1471-2164-10-199. PMID: 19400928

142. Ferrari D, Chiozzi P, Falzoni S, Dal Susino M, Melchiorri L, et al. (1997) Extracellular ATP triggers IL-1 beta release by activating the purinergic P2Z receptor of human macrophages. J Immunol 159: 1451-1458. PMID: 9233643

143. Ferrari D, Wesselborg S, Bauer MK, Schulze-Osthoff K (1997) Extracellular ATP activates transcription factor NF-kappaB through the P2Z purinoreceptor by selectively targeting NF-kappaB p65. J Cell Biol 139: 1635-1643. PMID: 9412459

144. Lee BH, Hwang DM, Palaniyar N, Grinstein S, Philpott DJ, et al. (2012) Activation of P2X(7) receptor by ATP plays an important role in regulating inflammatory responses during acute viral infection. PLoS One 7: e35812. doi: 10.1371/journal.pone.0035812. PMID: 22558229

145. Munoz A, Yazdi IK, Tang X, Rivera C, Taghipour N, et al. (2016) Localized inhibition of P2X7R at the spinal cord injury site improves neurogenic bladder dysfunction by decreasing urothelial P2X3R expression in rats. Life Sci 171: 60-67. doi: 10.1016/j.lfs.2016.12.017. PMID: 28039004

146. Goncalves RG, Gabrich L, Rosario A Jr, Takiya CM, Ferreira ML, et al. (2006) The role of purinergic P2X7 receptors in the inflammation and fibrosis of unilateral ureteral obstruction in mice. Kidney Int 70: 1599-1606. doi: 10.1038/sj.ki.5001804. PMID: 16969386

147. Odobasic D, Kitching AR, Holdsworth SR (2016) Neutrophil-Mediated Regulation of Innate and Adaptive Immunity: The Role of Myeloperoxidase. J Immunol Res 2016: 2349817-11. doi: 10.1155/2016/2349817. PMID: 26904693

148. Dell'Antonio G, Quattrini A, Cin ED, Fulgenzi A, Ferrero ME (2002) Relief of inflammatory pain in rats by local use of the selective P2X7 ATP receptor inhibitor, oxidized ATP. Arthritis Rheum 46: 3378-3385. doi: 10.1002/art.10678. PMID: 12483745

149. Chessell IP, Hatcher JP, Bountra C, Michel AD, Hughes JP, et al. (2005) Disruption of the P2X7 purinoceptor gene abolishes chronic inflammatory and neuropathic pain. Pain 114: 386-396. doi: 10.1016/j.pain.2005.01.002. PMID: 15777864

150. Yan Y, Bai J, Zhou X, Tang J, Jiang C, et al. (2015) P2X7 receptor inhibition protects against ischemic acute kidney injury in mice. Am J Physiol Cell Physiol 308: doi: 10.1152/ajpcell.00245.2014. PMID: 25588875

151. Wan P, Liu X, Xiong Y, Ren Y, Chen J, et al. (2016) Extracellular ATP mediates inflammatory responses in colitis via P2 x 7 receptor signaling. Sci Rep 6: 19108. doi: 10.1038/srep19108. PMID: 26739809