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Current Pharmacologic Approaches in Painful Bladder Research: An Update

Karl-Erik Andersson[1,2], Lori Birder[3]

[1]Institute for Regenerative Medicine, Wake Forest University School of Medicine, Winston Salem, NC, USA
[2]Institute of Laboratory Medicine, Lund University, Lund, Sweden
[3]Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

The symptoms of interstitial cystitis (IC)/bladder pain syndrome (BPS) may have multiple causes and involve many contributing factors. Traditional treatments (intravesical instillations) have had a primary focus on the bladder as origin of symptoms without adequately considering the potential influence of other local (pelvic) or systemic factors. Systemic pharmacological treatments have had modest success. A contributing factor to the low efficacy is the lack of phenotyping the patients. Individualized treatment based on is desirable, but further phenotype categorization is needed. There seems to be general agreement that IC is a unique disease and that BPS is a syndrome with multiple pathophysiologies, but this has so far not been not been well reflected in preclinical research with the aim of finding new pharmacological treatments. Current research approaches, including anti-nerve growth factor treatment, anti-tumor necrosis factor-α treatment, activation of SHIP1 (AQX-1125), and P2X3 receptor antagonists, and α_1-adrenoceptor antagonists are potential systemic treatments, implying that not only the bladder is exposed to the administered drug, which may be beneficial if the IC/BPS is a bladder manifestation of a systemic disease, or negative (adverse effects) if it is a local bladder condition. Local treatment approaches such as the antagonism of Toll-like receptors (which still is only experimental) and intravesical liposomes (with positive proof-of-concept), may have the advantages of a low number of systemic adverse effects, but cannot be expected to have effects on symptoms generated outside the bladder. Assessment of which of the treatment approaches discussed in this review that can be developed into useful therapies requires further studies.

Keywords: Nerve Growth Factor; Tumor Necrosis Factor-alpha; SHIP1; P2X3 Receptor; Toll-Like Receptors; Liposomes

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INTRODUCTION

In the search of targets for pharmacologic treatment of interstitial cystitis (IC)/bladder pain syndrome (BPS), there has traditionally been a primary focus on the bladder and lower urinary tract as origin of symptoms without adequately considering the potential influence of other local (pelvic) or systemic factors. Several epidemiological studies have shown that IC/BPS is commonly associated with other chronic pain conditions, including fibromyalgia, irritable bowel syndrome, and chronic fatigue syndrome, which suggests that IC/BPS may involve systemic pathophysiology, including changes in the central nervous system. Thus, it has to be decided whether to treat a local bladder disease or a systemic disease with bladder manifesta-

Corresponding author: Karl-Erik Andersson https://orcid.org/0000-0003-0665-3486
Institute of Laboratory Medicine, Lund University, 223 62 Lund, Sweden
E-mail: karl-erik.andersson@med.lu.se / Tel: +46-705522722
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tions. There may be multiple causes and contributing factors that can manifest in the symptoms of IC/BPS, and there may also be multiple patient subgroups or phenotypes. Recently some messages were delivered [1] that may give important directions for future research: (1) IC is a unique disease. (2) BPS is a syndrome, not a disease. (3) Patients should be phenotyped before treatment. Current research on possible targets for treatment IC/BPS, some of which are discussed in this review, reflects to some extent these messages.

METHODS

A search for literature concerning recent preclinical and clinical research on pharmacological targets relevant for treatment of IC/BPS was performed. The electronic databases PubMed, Google Scholar, and Scopus were used to identify relevant clinical and animal studies. Keywords were entered as medical subject headings (MeSH) or as text words. The Mesh terms were used in various combinations and usually included the terms chronic pelvic pain, bladder, afferent signalling, cystitis, IC/BPS, treatment, pharmacology. Search results were assessed for their overall relevance to this review.

EMERGING TARGETS

Pain during bladder filling is a perception and signalling from afferent nerves, particularly from the bladder, but also from other pelvic organs ("cross-talk"), can be expected to play an important role in its generation. It is therefore not surprising that most recent research is "bladder-centric," implying that it has a focus on possible targets within the bladder. However, systemic administration of a drug implies that not only bladder is exposed, which may be beneficial if the IC/BPS is a bladder manifestation of a systemic disease, or negative (adverse effects) if it is a local bladder condition.

ANTI-NERVE GROWTH FACTOR TREATMENT

Elevated levels of nerve growth factor (NGF) have been demonstrated in the bladder of various animal models of bladder inflammation, e.g., chemical, neurogenic, and immune-mediated [2]. In rats, acute intravesical administration of NGF produced sensitization of bladder afferents, resulting in increased frequency of micturition and reduced micturition threshold (as measured by cystometry). In patients with IC/BPS the bladder expression of NGF was found to be increased [3], and urine obtained from IC/BPS patients contained increased levels of the neurotrophin [4]. A recent meta-analysis reported that urinary NGF could be a useful biomarker for the differential diagnosis of IC/BPS and overactive bladder as well as a predictive biomarker to help guide treatments [5]. The results from preclinical and clinical studies would therefore suggest inhibition of the release or actions of NGF to be reasonable treatment approaches. In a phase II study [6], the effects of tanezumab, a monoclonal NGF neutralizing antibody, given intravenously, were studied in 64 IC/BPS patients (34 receiving tanezumab). At week 6 tanezumab produced a significant reduction from baseline in average daily pain score vs. placebo and a significantly higher proportion of patients on tanezumab responded as improved in the global response assessment. Tanezumab also significantly reduced urgency episode frequency vs. placebo, but had no significant effect on Interstitial Cystitis Symptom Index score, micturition frequency, or mean voided volume per micturition. Side effects included headache and paraesthesia. This study suggested a positive effect of the agent. However, serious adverse events were reported in an initial study of tanezumab in osteoarthritis, in which bone necrosis developed and total joint replacements were needed, and the agent was temporarily put on clinical hold [7]. After the clinical program was resumed in 2012, several studies on osteoarthritis have been performed, and in a recent meta-analysis tanezumab was shown to provide superior pain relief and improvement in physical function and patient's global assessment over placebo in knee and hip osteoarthritis patients, and tanezumab was generally well tolerated with acceptable adverse effects [8].

Fulranumab, another human monoclonal antibody directed against NGF, showed efficacy in patients with moderate-to-severe osteoarthritis pain [9]. Wang et al. [10] performed a multicenter, double-blind study on adult patients with IC/BPS. The study was terminated prematurely for the same reasons as tanezumab (see above), but 31 patients (of 70 patients targeted) were randomized, 17 to placebo and 14 to fulranumab, with 15 and 10 patients, respectively, receiving all 3 doses of double-blind treatment. There was no statistically significant difference between treatment groups for the primary endpoint (average daily pain intensity score). However, the authors did not exclude the possibility that the drug would provide clinical benefit in a larger study and/or specific populations [10]. This may be the case, but even if fulranumab was well tolerated in this study, in the osteoarthritis study by Sanga et al. [9], rapid progression

of osteoarthritis was observed as a safety signal.

NGF may be an important pathophysiological factor inducing pain-related symptom in IC/BPS. However, even if the results with tanezumab may provide proof-of-concept evidence that inhibition of NGF can be effective for the treatment of IC/BPS, potential safety issues related to systemic administration have to be clarified. This opens up for local approaches such as liposome-based intravesical therapy targeting NGF [11,12].

ANTI-TUMOR NECROSIS FACTOR-α TREATMENT

The tumor necrosis factor (TNF) is a proinflammatory cytokine released by immune cells which may be involved in acute and chronic inflammation in autoimmune diseases [13]. The TNF and TNF receptor superfamilies have a wide range of actions including promotion of cellular differentiation, survival, and production of inflammatory cytokines and chemokines [14,15]. Interestingly, Ogawa et al. [16] investigating genes responsible for ulcerative IC, found an increased mRNA expression of several genes related to immune and inflammatory responses in bladder urothelium of patients with ulcerative IC, including TNFSF14 (TNF ligand superfamily member 14), a lymphotoxin-like inducible protein that has an important role in the pathogenesis of autoimmune disease. Such changes were not found in patients with symptoms but without ulcerations.

Clinically, the anti-inflammatory effect of TNF blockade has been explored for the treatment various diseases [14,15]. Among several TNF antagonists, adalimumab, a fully human high-affinity, recombinant immunoglobulin G_1 anti-TNF monoclonal antibody [13], has been tested in IC/BPS patients [17]. Adalimumab has a high selectivity and affinity for TNF and reduces TNF-induced inflammatory responses.

Bosch [17] reported the results of a phase III, randomized, double-blind, placebo-controlled proof-of-concept study on the effects of adalimumab in 43 patients (21 receiving drug) with moderate to severe IC/BPS. The patients were given 80-mg subcutaneous adalimumab followed by 40 mg every 2 weeks or subcutaneous placebo for 12 weeks. Compared to baseline, adalimumab treatment resulted in a statistically significant improvement in outcome measures (O'Leary-Sant Interstitial Cystitis Symptom and Problem Indexes, Interstitial Cystitis Symptom Index, Interstitial Cystitis Problem Index, and Pelvic Pain, Urgency, Frequency Symptom Scale). After 12 weeks of treatment, 11 of 21 patients (53%) in the adalimumab group had a 50% or greater improvement in global response assessment. There were no significant adverse events. However, the drug failed to demonstrate positive proof-of-concept compared to placebo due to a significant placebo effect.

Anti-TNF therapeutics have been successful and changed the management of autoimmune diseases such as rheumatoid arthritis [18]. However, so far it is unclear if this drug principle can be developed to a useful treatment alternative for patients with IC/PBS, and further controlled studies are needed.

ANTAGONISTS OF TOLL-LIKE RECEPTORS

It is well established that there is an overlap between IC/BPS and known autoimmune disorders, such as Sjoegren syndrome, systemic lupus erythematosus (SLE), rheumatoid arthritis, and ulcerative colitis [19-21]. Toll-like receptors (TLRs) play a key role in the innate immune system and in biological defense mechanisms against external pathogens such as bacteria, fungus, and virus [22]. Among the TLRs demonstrated in humans, TLR7 is expressed not only in immune reactive cells but also neurons and glia cells. TLR7 is associated with the development and maintenance of inflammation and pain and has been considered to contribute to the development of several autoimmune diseases, such as SLE and Sjoegren syndrome [23,24].

Ichihara et al. [25] studied TLR7 expression in bladder biopsy specimens from patients with Hunner-type IC. They also explored the functional roles of TLR7 in bladder inflammation and nociception in mice. Ichihara et al. [25] found that the number of TLR7 immuno-reactive cells and the mRNA expression of TLR7 were significantly higher in IC specimens than in samples from controls without the disease. Loxoribine, a TLR7 agonist, instilled in the bladder of C57BL/6N female mice, induced edema, congestion, and inflammation, and significantly increased the TLR7-mRNA expression. The drug also significantly increased licking behaviour, voiding frequency, and afferent nerve activities associated with decreased single-voided volume and intercontraction interval of micturition. In addition, activation of TLR7 induced cystitis with sensory hyperactivity. Hydroxychloroquine, a TLR7 antagonist, instilled intravesically, reversed the loxoribine induced cystometric and voiding behavioural changes. It may be speculated if blocking the TLR7 pathway in the bladder of IC patients can be a new treatment approach. Further studies are needed to confirm and explore the translational impact of these observations.

AQX-1125, A MODULATOR OF IMMUNE/INFLAMMATORY PROCESSES

AQX-1125 represents a new pharmaceutical class of compounds [26], activators of SH2-containing inositol-5′-phosphatase 1 (SHIP1). SHIP1 is an intracellular protein whose expression is primarily restricted to cells of the haematopoietic lineage. It is a negative regulator of the PI3K pathway and suppresses intracellular signalling by catalysing the degradation of PIP3 to PI(3,4) P2 (PIP2), thus suppressing PIP3-mediated cellular effects. In addition to its catalytic function, SHIP1 can also act as a negative regulator of signalling through its interactions with other proteins [27]. SHIP1 is a negative regulator of many inflammatory signalling processes of the immune system, and has documented roles in controlling vital cell functions such as development, proliferation, activation, cytokine secretion and migration [28]. Activation of SHIP1 has an anti-inflammatory effect [29]. The highly restricted expression pattern of SHIP1 in addition to its ability to act as a negative regulator of immune cell signalling makes it a highly attractive target for the development of anti-inflammatory therapeutics, and pharmacological activation of SHIP1 has emerged as a novel approach for the therapy of various inflammatory diseases [26,29,30].

AQX-1125 has been studied in a short-term, phase II, randomized, placebo controlled study, which included patients with moderate to severe IC/BPS, some with Hunner lesions (Aquinox Trial) [31]. Thirty-seven IC/BPS women were treated with the compound and 32 with placebo. Women treated with AQX-1125 showed a significant reduction in bladder pain and improvement of symptoms at 6 weeks compared to placebo-treated women [31]. Thus, anti-inflammatory treatment targeting the PI3K pathway could potentially be a useful strategy for the treatment of IC/BPS patients, especially when bladder inflammatory changes such as Hunner lesions are identified in the bladder [16].

P2X3 RECEPTOR ANTAGONISTS

Bladder distension stimulates the urothelium to release factors, such as ATP, from its mucosal and serosal surfaces [32]. ATP then activates P2X3 receptors in bladder afferents to modulate bladder activity as evidenced by experimental studies of P2X3 knockout mice [33]. In IC/BPS, the urothelial expression of P2X3 is increased and the stimulatory ATP mechanism is up-regulated [34-37]. It seems therefore logical to expect that inhibition of ATP release or blockade of P2X3 receptors would be effective ways of blocking afferent activity involved in symptom generation.

AF-219 is an orally active small molecule antagonist at human P2X3-containing receptors that in a double-blind, placebo-controlled, 2-period, crossover study was effective as an antitussive [38]. Moldwin et al. [39] performed a placebo-controlled clinical trial with AF-219 in female IC/BPS patients, 36 treated with the drug and 38 with placebo. Patients treated with AF-219 for 4 weeks had improvement in the key symptoms of IC/BPS such as pain scores, urinary urgency and in global response assessment, compared to placebo-treated patients. There were 5 patients (4 in the AF-219 arm and 1 in the placebo arm) with Hunner lesions on cystoscopy. Thus, targeting the ATP and P2X receptor mechanism in the bladder may be a promising strategy for the treatment of IC/BPS with or without Hunner lesions.

REDUCTION OF SYMPATHETIC OVERACTIVITY AND STRESS

There is an increasing body of evidence suggesting that the sympathetic system is implicated in chronic painful conditions [40,41]. Studies have shown that α-adrenoceptors (ARs) increase pain in human disorders such as complex regional pain syndrome which exhibit a number of parallels to IC/BPS [42-45]. IC/BPS patients excrete high levels of urinary catecholamines [46,47], and a pathophysiologic role for a generalized cardiovascular and vasomotor autonomic nervous system abnormality in IC/BPS patients has been suggested. In a preliminary study, Chelimsky et al. [48] evaluated and compared the structural integrity of the autonomic nervous system in IC/BPS and control subjects, but found no differences, except that IC/BPS patients had higher baseline heart rate. In a follow-up study the same group compared IC/BPS patients with those suffering from another disorder, myofascial pelvic pain with pain in the pelvis unrelated to bladder state. A higher baseline heart rate was confirmed, and 6 of 36 IC/BPS patients (22%) were shown to have an autonomic neuropathy.

A link between chronic pain and the sympathetic system may be concluded from several animal experiments [49-52]. Charrua et al. [47] injected phenylephrine subcutaneously daily for 14 days in female rats and found increases in visceral pain, spinal Fos expression, bladder reflex activity, urinary spotting, and the number of expelled fecal pellets. The mucosa showed

urothelial thinning and increased immunoreactivity for caspase 3 and bax. Trypan blue staining suggesting increased urothelial permeability was only observed in phenylephrine treated animals, which also showed increased sympathetic nerve density and urinary noradrenaline levels. Based on their results, Charrua et al. [47] concluded that excessive adrenergic stimulation of the bladder may contribute to the pathophysiological mechanisms of BPS/IC. In a follow-up study, Matos et al. [53] showed that phenylephrine-induced morphological bladder changes, bladder overactivity, and L6 spinal cord Fos expression, were reversed by the highly selective α_{1A}-AR antagonist, silodosin, but not by naftopidil, a relatively selective antagonist for α_{1D}-ARs. Prazosin (which blocks all subtypes of α_1-ARs) had an intermediate effect. Pain elicited by phenylephrine could be elicited in wild-type, but not TRPV1 knockout, mice. Silodosin also reversed the enhanced contraction responses to capsaicin found phenylephrine-treated rats. Primary afferents can coexpress α-ARs and TRPV1 receptors [47,53], which may lead to interactions between sympathetic efferent nerves and nociceptive primary afferent nerves via a chemical cross-talk. When human urothelial cells (which express ARs) were treated with phenylephrine, ATP release, by mechanical stimulation or by lowering the thermal threshold of urothelial TRPV1, was enhanced. The authors suggested that activation of peripheral α_{1A}-ARs induces chronic visceral pain, probably through its interaction with TRPV1 and ATP release.

Chronic emotional stress may produce sensitization and enhanced excitability of afferent pathways innervating the lower urinary tract, and this is believed to play a role in the exacerbation and possibly the development of functional lower urinary tract disorders [54,55]. The water avoidance stress (WAS) in rodents reproduces signs of nociception and bladder changes seen in IC/BPS patients [56,57]. Matos et al. [57] explored the possible role of α1A-ARs in bladder pain and morphological changes induced by WAS in a group of female Wistar rats. A separate WAS group received silodosin daily. Lower abdominal pain was determined by performing sensitivity to Von Frey filaments, and bladder reflex activity was assessed by cystometry in anaesthetised animals. WAS increased urinary noradrenaline levels and bladder frequency was increased in WAS animals, and the mechanical pain threshold was decreased. These effects were reversed by silodosin. Morphological changes such as lymphocytic and mast cells infiltration in the mucosa and mild urothelial disruption seen in the WAS group were absent in WAS+silodosin group.

These results suggest that in rats α_{1A}-ARs have an important role in the appearance of bladder pain induced either by pharmacological stimulation or by WAS, and that α_{1A}-AR inhibition can prevent not only pain but underlying morphological changes. It is not surprising that α_1-AR agonist induced changes may be reversed by antagonists. However, WAS induced functional and morphological changes in the bladder probably have a more complex pathophysiology, and chronic stress can in addition to sympathetic overactivity, involve activation of the hypothalamic-pituitary axis, dysregulation of the serotonergic pathways, and central sensitization [55]. Nevertheless, the finding that selective α_{1A}-AR inhibition can improve both functional and morphological changes in WAS animals, is of potential translational significance. Since BPS/IC patients present high blood levels of noradrenaline [46,47], α_{1A}-AR stimulation may be an additional trigger for bladder dysfunction in these patients. The response to treatment with α_{1A}-AR antagonists in patients with IC/BPS would be worthwhile exploring.

INTRAVESICAL LIPOSOMES

Liposomes are lipid vesicles composed of phospholipid bilayers surrounding an aqueous core [58]. The lipidic bilayer structure of liposomes facilitates their adherence to the apical membrane surface of luminal cells in the bladder. Liposomes are taken up by urothelial cells through clathrin-mediated endocytosis, and their vesicular shape allows them to use the endocytosis uptake machinery after instillation [12]. Empty liposomes can protect damaged urothelium and have shown therapeutic benefits for IC/BPS patients [12]. In addition, liposomes can carry various drugs to penetrate urothelium and modulate afferent neurotransmission [12]. The mechanism of action for empty liposomes in the bladder was suggested to involve a combination of a physical coating on the urothelium, and a drug-like action that promotes repair and regeneration of the bladder lining [12]. The primary constituent current used for instillation of liposome into bladders of IC patients is sphingomyelin, which has an anti-inflammatory effect.

The first clinical study was performed by Chuang et al. [59] who compared the response of intravesical liposome therapy to that of oral pentosan polysulfate. Twenty-four patients with moderate IC/BPS were prospectively included in the study, and 12 of them were given 80 mg of liposomal therapy instilled once weekly for 4 weeks. The other 12 patients received 300 mg of oral pentosan polysulfate daily. Outcomes were measured

using the O'Leary Sant score, pain assessment scale and global response assessment at 4 and 8 weeks posttreatment. Statistically significant decreases in pain, urgency and the O'Leary-Sant symptom score were noted in the liposome group at 4 weeks, which remained at 8 weeks. Urinary frequency and nocturia scores were comparable to the pentosan polysulfate group. There were no side effects, which were confirmed in a later study including 17 patients [60]. Peters et al. [61] followed 14 IC/BPS patients (11 women, 3 men) who underwent weekly instillations of liposomes for 4 weeks. Efficacy measurements included pain visual analog scales, voiding diaries, global response assessments, and O'Leary-Sant Interstitial Cystitis Symptom and Problem Indices. Cystoscopic improvements were noted in three patients, no change in 10 and worsening in 1 patient. No adverse side effects were noted and outcomes were positive with improvements in pain, urgency, and overall symptoms scores. Improvements in urgency were sustained at 8 weeks. Although pain scores significantly improved at 4 weeks, they were found to be back to baseline at 8 weeks.

Intravesical liposome therapy, both with empty liposomes but particularly with liposomes carrying active drugs, seems to be a promising approach for treatment of IC/BPS patients. However, effectiveness has to be assessed in large randomized controlled trials.

SUMMARY AND CONCLUSIONS

Inflammation and signalling from afferent nerves in the bladder and other pelvic organs, can be expected to play an important role in the symptoms of IC/BPS. Current research approaches including anti-NGF treatment, anti-TNF-α treatment, activation of SHIP1 (AQX-1125), P2X3 receptor antagonists, and α_1-AR antagonists, despite having bladder-centric rationale, are potential systemic treatments which implies that they could be expected to have effects both on the bladder and on non-bladder manifestations of IC/BPS. Local treatment approaches such as the antagonism of TLRs (which still is only experimental) and intravesical liposomes (with positive proof-of-concept), may have the advantages of a low number of systemic adverse effects, but cannot be expected to have effects on symptoms generated outside the bladder. Individualized treatment based on phenotype (IC is a separate disease, BPS a syndrome) seems rational. However, assessment of which of the treatment approaches discussed that can be developed into useful therapies requires further studies.

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