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Reviewing Interstitial Cystitis Models and Treatments: A Focus on the Urothelium

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Abstract

Context: Interstitial cystitis is a multifactorial chronic and debilitating disease which is commonly associated with pain localized in the bladder region, increased urinary frequency, urgency and nocturia. Due to the high prevalence of pain in the population affected more recently the condition is often referred to as interstitial cystitis / bladder pain syndrome (IC/BPS).
Evidence Acquisition: Although IC presents with many different symptoms, researchers have formulated three different theories, which are not mutually exclusive, to explain IC pathology: the first is related to the alteration of the proteoglycan and protein junction composition, structure and presence in the urothelium. The second is an immune induced IC resulting from an increased number of activated mast cells in the bladder internal layers, such as detrusor muscle (DM), and mucosa/submucosa. The third type, which is closely related to the second one, is due to a sensory nerve sensitization as an effect of neurotrophic factors molecule release.
Results: Previous classification has been mirrored in the development of various *in vitro* and *in vivo* disease models created to mimic IC, as well as in the available therapies used to treat the condition to date. Conclusions: This review will summarize the most recent advances in the field, related to the different causative factors contributing to the development of the condition, the *in vivo* models used as well as the evidence they provide in advancing our knowledge and their limitations. The focus will be on works reporting on IC in the domain related to alterations in proteoglycans and cellular junction, specifically their composition, structure and appearance in the urothelium, and also discuss present and future therapies.
Conclusions: This review will summarize the most recent advances in the field, related to the different causative factors contributing to the development of the condition, the *in vivo* models used as well as the evidence they provide in advancing our knowledge and their limitations. The focus will be on works reporting on IC in the domain related to alterations in proteoglycans and cellular junction, specifically their composition, structure and appearance in the urothelium, and also discuss present and future therapies.

Keywords: Intestitial Cystitis/Bladder Pain Syndrome, Bladder, Bladder, Glycosaminoglycan, Pain

1. Context

Interstitial cystitis (IC) is a chronic and debilitating disease which generally causes pain localized in the pelvic region and occurs along with urinary frequency, urgency and nocturia symptoms. Many etiological factors have been correlated with IC such as infection, autoimmunity, inflammatory processes, local neuronal dysfunction, bladder tissue abnormality and toxins, highlighting the multifactorial nature of this disease (1).

Because of its multifactorial etiology and symptoms, IC's epidemiology is still not clear. An attempt to examine the epidemiology of IC in a Finnish population of 1,000,000 people was carried out in 1975. This study reported 10.6 cases per 100,000 people, with an average of 18.1 cases per 100,000 in females (2). The high incidence within the female population was recently confirmed by another population based study (3), and also it was reported that the disease is more common in caucasians than in other races (4). The marked geographical differences in incidence may also reflect a difference in diagnostic criteria applied in each country, thus the national institute of diabetes and digestive and kidney disease (NIDDK) has recommended a set of criteria for IC diagnosis; (5) however the criteria developed have not been as helpful as originally intended (6).

The first reports of the symptoms related to IC go as far back as 1836, where the condition was referred as "tic douloureux", soon after Alexander Skene coined the term

"interstitial cystitis" to describe bladder disturbances involving chronic inflammatory and lesions (7). In 1915 Guy Hunner characterized these lesions, describing the presence of red bleeding areas on the bladder wall, which since then have been known as Hunner's lesions (8). Thus, in the fifties of the twentieth century, Hand et al. (1949) thoroughly studied the nature of the pathologic condition related to IC by using endoscopic and histologic techniques (9). However, since Hunner's characterization, numerous cases of IC with no evidence of bladder wall lesions, and pain related to bladder filling and emptying have been reported. This suggested the presence of at least two clinical conditions, ulcerative and non-ulcerative IC, depending on the presence or absence of Hunner's ulcers. Ulcerative IC was most common in older people, presenting with increased day and night-time urinary frequency, histological changes and diffuse bladder inflammation; by contrast, non-ulcerative IC was commonly associated with diffuse and often systemic syndromes, such as fibromyalgia (FM), chronic fatigue syndrome (CFS), irritable bowel syndrome (IBS), chronic pelvic pain (CPP), temporomandibular joint disorder (TMD), migraine, vulvodynia, low back pain, sicca syndrome, allergies, asthma, anxiety, and depression and can be alternatively categorized as a functional somatic syndrome (10). The differentiation of the two subtypes was also supported at least in part by the fact that patients with the ulcerative sub-type responded quite well to local therapies while in contrast patients with the non-ulcerative responded less well. Recent studies suggest that the non-ulcerative sub-type of IC is the result of a problem related to alteration in autonomic nervous system function, which connects central nervous system to terminal organs (11). Thus the international continence society (ICS) coined the term "bladder pain syndrome" (BPS) to distinguish between a suprapubic pain related to bladder filling, accompanied by other symptoms such as increased daytime and night-time frequency, in the absence of proven urinary infection or other obvious pathology and IC in which typical cystoscopic and urothelial histologic features are present (12) (Figure 1).

However, because of its multifactorial nature and despite the enormous efforts made to find a definitive cure, IC is still causing pain and distress to millions of people around the world, thus, needing more attention by the research community.

In this review, I focus on works reporting on IC in the domain related to alterations in proteoglycans and cellular junction. I summarize the recent advances in the field, related to the different causative factors contributing to its development. I also review the *in vivo* models used and the evidence they provide in advancing our knowledge, as well as highlight their limitations, and finally, I take into consideration present and future therapies.

2. Evidence Acquisition

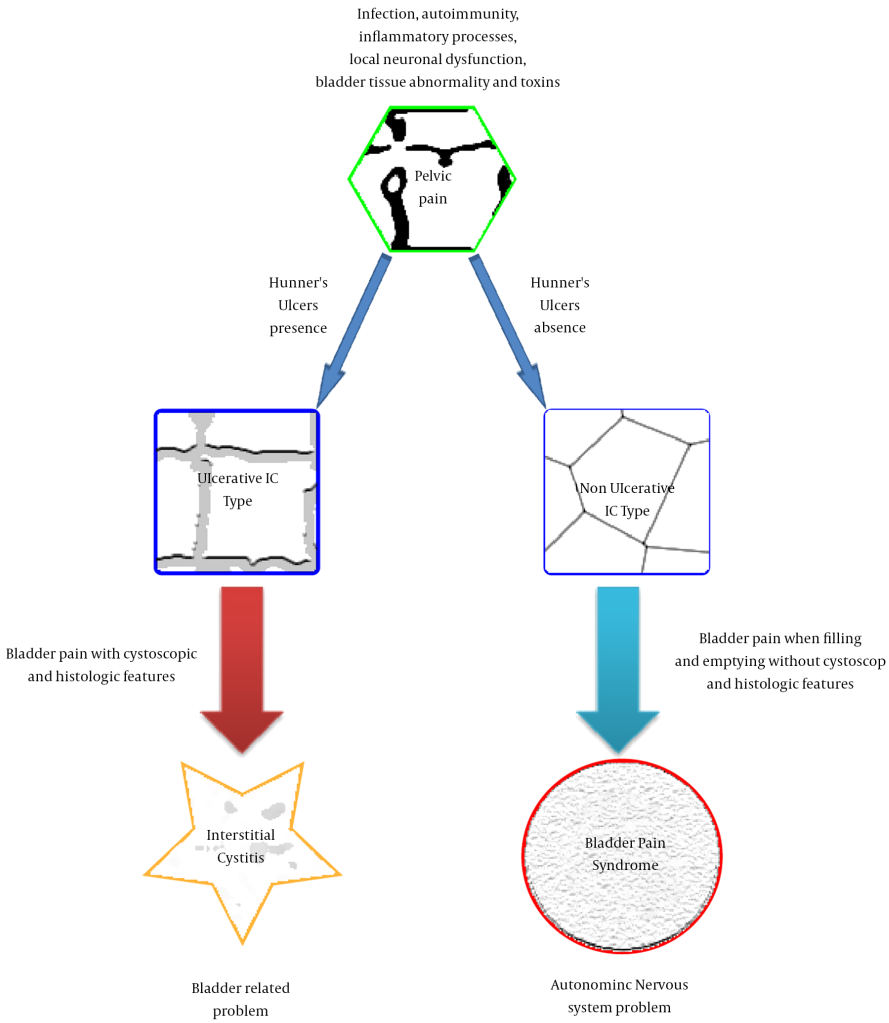
PubMed was used searching for reviews and research articles on interstitial cystitis treatments and models. The resulting publications (134) were reviewed for articles that met our inclusion and exclusion criteria. I included all those articles (79) which referenced treatments used in case of alterations in proteoglycans composition of the urothelium, within these criteria we included products already in commerce as well as new compounds not yet tested in clinical trials. Animal models were selected mainly, but not only, because of their relation to the alteration of the proteoglycans composition in the urothelium. Excluded articles were those that discussed IC related syndromes (Bladder Pain syndrome) or those mainly related to the disease management.

2.1. Bladder Structure and Function

Macroscopically the bladder is a hollow musco-elastic organ which collects the urine; it is composed of 4 different tissue layers: The serosa, which partially derive from the peritoneum; the muscular (detrusor muscle) consisting of 3 layers preferentially arranged in a layer-specific manner, longitudinal, circular and again longitudinal. This is followed by the sub-mucosa which connects the muscular layer to the mucosa (Figure 2). The most internal layer of the bladder, the mucosa, is likewise composed of different sub-layers. The outermost layer of the mucosa is called the lamina propria, rich in connective tissue interspersed with blood vessels, nerves, and in some regions, glands.

The structure and cell composition of the lamina propria is crucial for the coordination of the bladder filling, and it has an additional important integrative role regulating the signal transduction from and to the central nervous system (13). Moving inward, we find the basement membrane, a layer of extracellular material which acts as a filtration barrier and supports structure for the mucosal layer. The innermost layer is the urothelium, an epithelial layer which does not contain blood or lymphatic vessels. Microscopically, the urothelium is a transitional epithelial tissue also composed of different layers: a basal cell layer attached to the basement membrane, an intermediate layer, and a superficial or apical layer composed of large hexagonal cells (diameters of 25 - 250 μm) known as "umbrella cells" (Figure 3) (14). The urothelium is a functional epithelium acting as an impermeable barrier which prevents the infiltration of urinary solutes into the underlying tissue layers. Urothelial integrity is maintained as result

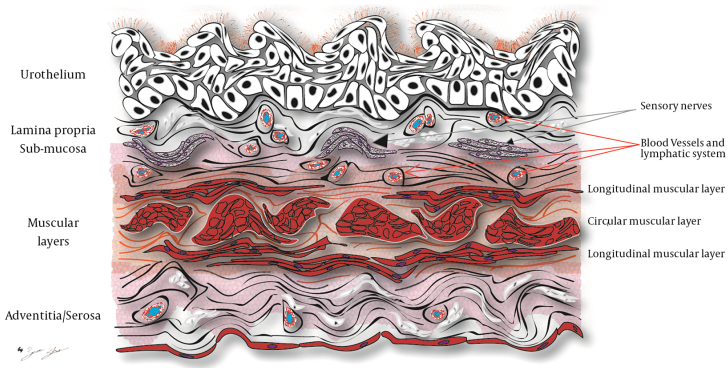
Figure 1. Interstitial Cystitis and Bladder Pain Syndrome



The term Bladder Pain syndrome was coined to distinguish between a pubic pain related to bladder filling, accompanied by other symptoms such as increased daytime and night-time frequency, in the absence of proven urinary infection or other obvious pathology and the IC in which typical cystoscopic and histologic features are present.

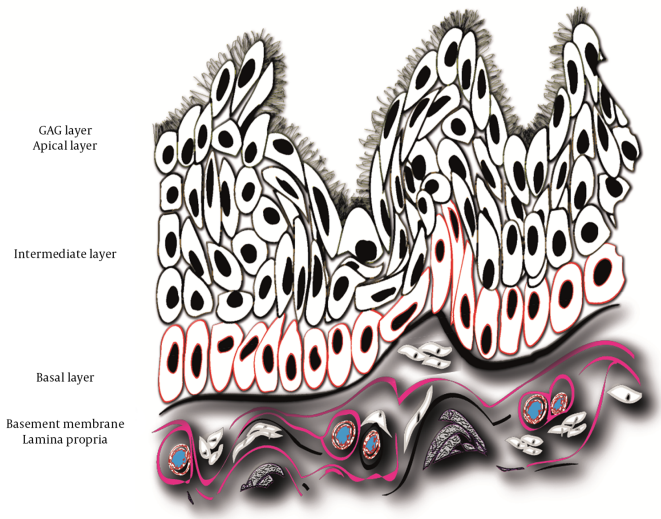
of a complex and balanced process involving migration, proliferation and differentiation, influenced by a range of factor including epithelial growth factor (EGF), platelet derived growth factor (PDGF), as well as retinoic acid (RA) (15). The structural and functional integrity of the urothelium is supported by intracellular protein complexes called tight junctions. These tight junctions are assembled in hexagonal plaques within the superficial umbrella cell layers and contribute to the urothelial barrier function by forming a unique asymmetric unit membrane (AUM). The proteins comprising these junctional complexes include common junctional proteins such as occludins, claudins and urothelial specific proteins such as uroplakins (16). The intact urothelium is also protected from the potentially toxic luminal environment of the bladder by a glycosaminoglycan (GAG) or mucin layer covering the umbrella cells. This layer has been demonstrated to be a key player in the barrier effect of the urothelium against microorganisms, carcinogens and toxic substances in the urine (13, 17). In addition, the lipid profile of the urothelium which is unusually rich in cholesterol, phosphatidylcholine, phosphatidilethanolammine and cerebroside, is also involved in the maintenance and restoration bladder barrier function (13).

Figure 2. Bladder Structure



The bladder is a hollow musco-elastic organ which collects the urine, composed of 4 different tissue layers: the adventitia/serosa, muscular layer, sub-mucosa and the mucosa.

Figure 3. Bladder Mucosa Structure



The most internal layer of the bladder, the mucosa, is likewise composed of different sub-layers, a basal layer, connected through the basement membrane to the lamina propria, an intermediate layer, and an apical layer, followed by the GAG external layer.

Even if the principal function of the urothelium is to prevent contact between the potentially harmful urine components and the lamina propria; it has also been demonstrated that these cells have nociceptive and mechanoceptive neural properties. Different types of stimulation (chemical, thermal, or mechanical stimuli) lead to the activation of urothelial cells, inducing the secretion of different mediators or neurotransmitters which, through the lamina propria, can influence nerve activity, detrusor muscle contraction and immuno-system function (13).

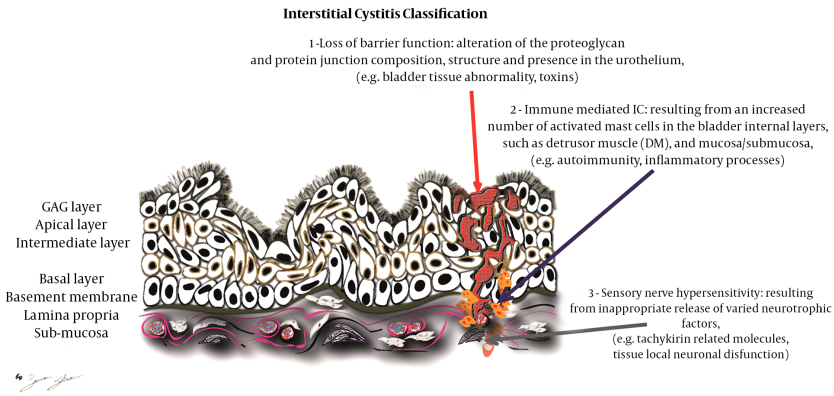
2.2. IC Classification

Despite the many and varied presentation of IC symptoms three different, not mutually exclusive, theories have been formulated to explain IC pathology (Figure 4).

1- Loss of barrier function: Alteration of the proteoglycan and protein junction composition, structure and presence in the urothelium, (e.g. bladder tissue abnormality, toxins) (17).

2- Immune mediated IC: Resulting from an increased number of activated mast cells in the bladder internal layers, such as detrusor muscle (DM), and mucosa/submucosa, (e.g. autoimmunity, inflammatory

Figure 4. IC Classification



Three different, not mutually exclusive, theories have been formulated to explain IC pathology and this is also mirrored in the animal models which have been created to mimic IC disease. 1, Loss of barrier function: Alteration of the proteoglycan and protein junction composition, structure and presence in the urothelium, (e.g. of bladder tissue abnormality, toxins). 2, Immune mediated IC: Resulting from an increased number of activated mast cells in the bladder internal layers, such as detrusor muscle (DM), and mucosa/submucosa, (e.g. autoimmunity, inflammatory processes). 3, Sensory nerve hypersensitivity: resulting from inappropriate release of varied neurotrophic factors, (e.g. tachykirin related molecules, tissue local neuronal disfunction).

processes) (18).

3- Sensory nerve hypersensitivity: resulting from inappropriate release of varied neurotrophic factors, (e.g. tachykirin related molecules, tissue local neuronal dysfunction) (18).

Previous classification is mirrored in the animal models which have been created to mimic IC disease.

2.3. Induced IC Animal Models with Effects on GAGs Depletion and Urothelial Permeability

The urothelium is an impermeable barrier which prevents the interaction of the urinary solutes with the underlying tissues. Its integrity is maintained as result of an equilibrated process of migration, proliferation and differentiation (15). Tight junctions contribute to the urothelial barrier function, among those sealing proteins are the occludins, claudins as well as uroplakins (15, 16). The glycosaminoglycan layer is also involved in the barrier effect of the urothelium against microorganisms, carcinogens and toxic substances in the urine (13, 17).

Chemical agents are commonly used to induce IC in animal models with the intention to directly degrade the GAG layer and alter intercellular tight junctions, thus reducing barrier effectiveness (19). Cyclophosphamide (CyP) is commonly used for the treatment of cancers as well as for various autoimmune diseases, however, many patients manifested an unpleasant IC like symptoms as side effect afterward the therapy (20).

As a consequence, the most common chemical irritant used to induce IC in animals is CyP. It is injected intraperitoneally and thus transformed by passing through the liver in acrolein which accumulates into the bladder causing urothelial damage, edema, accumulation of leukocytes in bladder tissue and hemorrhage (21).

It was reported that acrolein negative effect causes a reduction of endogenous glutathione which, by contrary, increases the generation of free radicals, such as superoxide anion and hydroxyl radical, thus lipid peroxidation and cell damage (22).

Another chemical agent is protamine sulfate (PS), originally isolated from the sperm of salmon. It has a highly cationic charge that permits itself to bind to heparin forming a stable ion pair. The bond between PS and heparin leads to the inactivation of heparin, reversing its anticoagulant effect (23). PS instillation directly into the bladder is more aggressive than CyP and causes extensive damage to the surface GAG layer of urothelial cells, decreases trans-epithelial resistance, thereby, induces a rapid epithelial desquamation. Unfortunately the treatment with PS does not induce bladder hypersensitivity and hyperactivity, two common IC symptoms (24). The effect of these compounds has been demonstrated at the molecular level, where the appearance of claudin-1, zonula occludens-1, (ZO-1), occludin-1, are all modified in the bladders of both CyP and PS treated of Sprague Dawley rodent models. Other chemical stripping approaches have also been utilized, for example: acetone, xylene, acidic solutions and mustard oil instillation or injection, as well as electrical stimulation, have been proved to deplete the GAG layer and thus negatively influence urothelium barrier function by increasing permeability resulting in IC symptoms in numerous animal models (25). A recent study presented evidence

that mouse bladder infused with 3% acetic acid followed by repeated treatment with a synthetic form of the antiproliferative factor (APF) results in IC related symptoms. These symptoms included urothelial thinning/ulceration, reduced urothelial repair, and decreased expression of uroplakin-III (UPIII), ZO-1 in the regenerating epithelium (26). Also, hyaluronidase instillation into the rat bladder resulted in a reduced urothelium thickness, chronic inflammation and increased infiltration of activated mast cells, as well as fibrosis. In addition, molecular analysis confirmed an abnormally low expression for uroplakin III and ZO-1 in animals treated with hyaluronidase alone, with respect to the controls (27).

Continuing, it was also found that the intravesical instillation of cathelicidin, an antimicrobial peptide, and LL-37, C-terminal peptide fragment of the human cathelicidin, induces Hunner's lesions in the mucosa, acute inflammation, marked edema in all layers of the bladder and infiltration of polymorphonuclear leukocytes in healthy animal models (28). Other researchers also demonstrated that moderate stress, induced by exposing mice to constant illumination for 96 hours, resulted in desquamation of superficial and intermediate urothelial cells (29).

2.4. Activated Mast Cells and the Immune Response in Bladder IC

Mast cells contribute to allergic and hypersensitivity reactions; in addition, these cells respond to non-immunologic stimuli derived from kinins, neuropeptides (such as neurotensin, somatostatin, substance P, neuropeptide Y and acetylcholine). Many studies have reported increased numbers of mast cells in the bladders of patients with IC where their degranulation releases granule-stored molecules (heparin, histamine, proteases, phospholipases, chemotactic substances, and cytokines), as well as the synthesis of cytokines, (prostaglandins, nitric oxide leukotrienes, platelet-activating factor, and interleukin), vasodilatory molecules, the vasoactive intestinal peptide and tumor necrosis factor (30).

Experimentally immune-induced cystitis (EIC) can be obtained by immunizing different mice strains (BALB/cAN and SWXJ) with bladder homogenate from syngeneic animals (31). This induces an immune IC with edema, fibrosis, perivascular lymphocytic infiltrations and deposition of mast cells in the muscular layer of the bladder in BALB/cAN mouse (31).

In SWXJ mice, the same immune challenge generates a similar immune IC with increased urination frequency, decreased volume per void, as well as lymphocytic infiltration and thickening of the lamina propria (32). A similar model with increased urinary frequency, mast cell accumulation and vascular congestion, has also been reported in Lewis rats by immunization with bladder homogenate (33). Recent studies suggest the involvement of T helper cells as key players in bladder homogenate-induced IC models (34). Another EIC model was generated by immunizing SWXJ mice with a recombinant uroplakin II, which is abundantly expressed in urothelial cell membranes (35). The symptoms exhibited by those mice were an increased urination frequency, decreased volume per void, thus, and an increased infiltration of T cells in the bladder. In contrast to models with effects on GAGs depletion and urothelium permeability, but as in the EIC models previously discussed, visceral pain was not reported in this model.

The sensitization to ovalbumin (OVA), as result of multiple intraperitoneal injections of the antigen followed by injection of OVA into the bladder, also leads to the development of experimentally induced cystitis in guinea pigs associated with increased urothelium permeability symptoms (36).

Physical and psychological acute stress factors have also been shown to trigger mast cells activity in animal models. Restraint and cold stress were used in one study and resulted in IC related symptoms including edema, leukocyte invasion, and mast cell degranulation in the bladder (37).

Transgenic mice have been also created to mimic experimental autoimmune cystitis however; despite the fact that many of the symptoms associated with IC disease were reported, such models do not represent the spectrum of alterations in the bladder related urodynamic changes and pain (38).

2.5. Increased Neural Sensitization and Neuro-Derived Substances in Bladder IC

Neurogenic IC results from pathologic over-activation of afferent/efferent primary sensory nerves (C-fibers). In the bladder the afferent function controls the micturition reflex, and pain transmission; the efferent function, among others, is related to the induction of the smooth muscle contraction, characteristic of neurogenic IC. C-fibers are considered polymodal because they can react to various stimuli (e.g. thermal, or mechanical, or chemical), caused by the release of different neurotransmitters (e.g. substance P, neurokinin A and B, neuropeptide K, neuropeptide γ, hemokin 1, endokinin A and B as well as calcitonin gene-related peptide (CGRP)) (39). As a result, central nervous system (CNS) receives the afferent C-fibers signals from the stimulated peripheral nociceptors, triggering central mechanisms that amplify and perpetuate the effect of the peripheral sensory nerve input (40). Simplifying, the mechanism behind neurogeic IC is the result of a neuronal sensitization as an effect of secreted neurotrophic factors, neurotransmitters or neuropeptides. In

addition to this, it was also reported that the neurotrophic factors involved in neurogenic IC are also able to stimulate the mast cells activation and proliferation (41). The close relationship between auto-immune and neuro-derived IC is supported by the fact that the secretion of vasoactive, nociceptive, and pro-inflammatory molecules from the activated mast cells contributes to increased neuropathic pain in IC patients (18, 42). Jerde TJ. et al. (2000) demonstrated that intravesical administration of substance P or bacterial endotoxin LPS results in the cystitis symptoms with pain localized in the bladder region, bladder neutrophil invasion, hemorrhage and edema (42). A neurogenic IC with bladder inflammation was also caused in different rodent models by injecting pseudorabies virus (PRV) into the dorsalis tail muscle (43). PRV induced cystitis symptoms with tumor necrosis factor alpha-dependent mast cell infiltration into the lamina propria, and histamine mediated pelvic pain. Also, the pathophysiology involved the presence of lesions in the urothelium with a decreased transepithelial resistance (44).

3. Results

3.1. IC Therapies

Traditionally light to mild manifestations of IC in humans are treated empirically by providing support and understanding to the patient, aiming to control or ameliorate symptoms, while mild to severe cases are treated by simple avoidance of the consumption of some foodstuffs, such as caffeine, alcohol or acidic foods (45). However, now based on the development of the identification of the different sub group, and considering the different histo-pathologic clinicians are better equipped to treat patients with specific pharmacologic drugs, developed based on in vivo animal models (18). In fact, IC due to an increased urothelial permeability, as a result of GAGs degradation, accompanied with a reduced presence of junctional proteins in the epithelium, is differentially treated with respect to immunologically stimulated IC, as result of an increased presence of mast cells in the bladder layers, and IC derived from bladder nerve sensitization, due to the neurotrophic factors release and over sensitization. More recently common therapies to treat IC involve the bladder instillation with drugs and oral drugs and of course patients in the first instance prefer oral therapy to intravesical therapy directly into the bladder; however, failure of this approach requires the most invasive treatment and today a plethora of treatments is being used.

3.2. Mast Cells Inflammation and Sensory Nerve Sensitization Drug Treatments

Since Hunner's IC characterization was performed, numerous cases of IC with no evidence of bladder wall lesions were confirmed (10). As already said, ICs with mast cells invasion and inflammation, as well as IC due to sensory nerve sensitization show both the presence and the absence of Hunner's ulcers, but their incidence is low. However, the fact that the patients with the ulcerative sub-type respond quite well to local therapies (e.g. bladder instillation) raised the suspicion that the non-ulcerative one represents a different specific systemic disease called bladder pain syndromes (BPS) (12, 46). Pain in BPS patient has neuropathic as well as somatic components and it is the direct consequence of both direct nerves damage or prolonged stimulation of the bladder nerves. Therefore, BPS can be treated just by using drugs or treatments against those general symptoms, such as pain and inflammation. Somatic pain is generally treated with opioids and non-steroidal anti-inflammatory drugs (NSAIDs), while neuropathic pain is usually treated with anti-epileptic and antidepressant drugs. Inflammation related BPSs have been successfully treated with inhibitors of bladder mast cell secretion cimetidine, cromolyn, hydroxyzine, indolinone derivatives, quercetin, capsaicin, botulinum toxin; all those drugs have benefits and disadvantages in the treatment of BPS patients and are well summarized in Table 1 (13). However, these therapies do not solve the problem when IC with a damaged urothelium is the cause as well as Hunner's ulcers are present.

3.3. GAGs Depletion and Urothelium Permeability, Drug Treatments

Different functional and structural abnormalities have been found in epithelial, endothelial, and in the detrusor muscle of the bladder of IC patients with ulcers, however, the most common pathological evidence of IC is the reduced thickness and functionality of the bladder urothelium. Many studies have also found that IC is caused by a decreased regenerative potential of bladder epithelial cells (47), others also confirmed that urothelial cells derived from IC patients display an impaired cell proliferation and increased cell permeability. All these conclusions have been confirmed at molecular level by the fact that cell cycle/proliferation related proteins (e.g. cyclin D1), as well as tight junction proteins expression is reduced in the bladder epithelial cells of IC patients (15).

Additionally, the GAGs on the top of the umbrella cells have been demonstrated to be a key player in the barrier effect of the urothelium against microorganisms, carcinogens and toxic substances in the urine. In fact, alterations

Table 1. Table 1

Therapeutic indication	Treatment	Mechanism	Adverse Effects
Light to mild manifestations of IC	Support and understanding to the patient	General behavior	None known
Mild to severe manifestations of IC in humans	Avoidance of the consumption of some foodstuffs, such as caffeine, alcohol or acidic foods	Alimentary behavior	None known
Mast cells inflammation	Cimetidine	Anti-inflammatory action	Impotence (reversible)
	Cromolyn		GI upset
	Hydroxyzine		Sedation
	Quercetin		Not reported
	Capsaicin		None known
	Botulinum toxin		Mild irritation
Somatic pain	Opioids	Analgesic action	Retention, addiction
Somatic pain	Non-steroidal anti-inflammatory drugs (NSAIDs)	Analgesic action	Ulcers, bleeding
Neuropathic pain	Anti-epileptic drugs	Analgesic action	Cognitive impairment
Neuropathic pain	Anti-depressant drugs	Analgesic action	Sedation
GAGs depletion and urothelium permeability	Dimethyl sulfoxide	Mucosal surface protector	Garlic-like body odor, initial pain
GAGs depletion and urothelium permeability	Heparin	Mucosal surface protector	Bleeding
GAGs depletion and urothelium permeability	Pentosan-polysulfate	Mucosal surface protector	GI upset, alopecia (reversible)
GAGs depletion and urothelium permeability	Prostaglandin E_1 analogue	Mucosal surface protector	Diarrhea
GAGs depletion and urothelium permeability	Chondroitin sulphate	Mucosal surface protector	None known
GAGs depletion and urothelium permeability	Hyaluronic acid	Mucosal surface protector	None known
GAGs depletion and urothelium permeability	Liposomes	Mucosal surface protector	None known
GAGs depletion and urothelium permeability	Hydrodistension and hyaluronic acid	Mucosal surface protector	None known
GAGs depletion and urothelium permeability	Intravesical instillation of HA + alkalinized lidocaine (AL)	Mucosal surface protector	None known
GAGs depletion and urothelium permeability	Intravesical instillation of heparin + alkalinized lidocaine (AL)	Mucosal surface protector + analgesic effect	None known
GAGs depletion and urothelium permeability	Sodium hyaluronate and chondroitin sulfate	Mucosal surface protectors	None known

in the GAG component of the protective mucin coating of the bladder urothelium permits urine substances, such as potassium, to penetrate the bladder wall, resulting in mast cell activation, inflammation, and sensory-nerve depolarisation (13, 17).

Mucosal surface protectors (such as Dimethyl sulfoxide (DMSO), heparin, pentosane-polysulfate (PPS), prostaglandin E_1 analogue, chondroitin sulfate and high molecular weight and low molecular weight hyaluronic acid) have been demonstrated to be helpful in relieving IC symptoms. The “extrema ratio” behind these treatments is that the exogenously supplied GAGs help to restore the defective GAG layer by coating and/or inducing the bladder epithelium to de novo produce a new GAG layer. More recent evidence suggests that the beneficial effect of the mucosal surface protectors (such as heparin, PPS, chondroitin sulphate, high molecular weight hyaluronic acid) may also be due at least in part to inhibition of mast

cell activation (48).

3.3.1. Dimethyl Sulfoxide

Dimethyl sulfoxide is an FDA approved treatment commonly used, since the sixties, as an intravesical therapy for ulcerative and non-ulcerative IC (49). It has been demonstrated that 50% (v/v) solution of DMSO has an anti-inflammatory, reactive oxygen scavenger action and easily crosses membranes. In addition, it has analgesic properties due to the impairment of the C-fibers signaling and acts as local anesthetic (50). It is well known that following DMSO instillation patients experience an initial flare up of pain which then subsides. In vitro studies on bladder tissue strips revealed the mechanism of action of DMSO confirming that initially DMSO causes urothelial barrier damage and cell leakage with consequent neural mediator release, such as ATP, acetylcholine, PGE2, nitric oxide and the urothelial-derived inhibitory factor (UDIF). Such an increased efflux of cytosolic mediators is the cause of the initial pain increase; however, this leads to their depletion followed by a relief effect as result of repeated DMSO instillation treatments (51). Side effects for DMSO therapy include a garlic-like body odor and, as already said, the DMSO instillation is painful. Thus, clinicians usually pre-treat or co-treat patients with local anesthetic into the bladder; nevertheless, many clinicians tend to prefer intravesical DMSO to heparin instillation (52) (Table 1).

3.3.2. Heparin

Heparin, like DMSO, is an FDA approved drug for the treatment of IC, its action is principally due to the protective properties. It provides to the bladder by affecting the GAG mucosal layer. Additionally it has an inhibitory effect on bladder mast cells granules (53). However, nowadays an increasing number of clinicians are substituting intravesical heparin treatment for DMSO (52) (Table 1).

3.3.3. Pentosan-Polysulfate

The semi-synthetic molecule PPS is a sulfated polysaccharide, similar to the GAGs which cover the urothelium, while also having anticoagulant and fibrinolytic properties. Results from clinical studies indicate that pentosane-polysulfate is able to replace damaged parts of the urothelial GAG layer, preventing contact between urine solutes and the underlying urothelium, as well as reducing bladder permeability (54). The treatment with PPS has demonstrated efficacy in IC animal models induced with cyclophosphamide (55). In addition, in vitro studies showed that nuclear factor-κB, a nuclear transcription factor involved in inflammatory response, is inhibited by PPS (56). However, pentosan-polysulfate produces side effects such as headache, rash, dizziness, diarrhea, dyspepsia, abdominal pain, hair loss (reversible), and liver function abnormalities (52) (Table 1).

3.3.4. Prostaglandin E1 Analogue

Prostaglandin E_1 analogue is normally used as a gastrointestinal protective molecule and it was also demonstrated to be helpful against IC symptoms because it affects various immunologic cascades. It has been shown to inhibit platelet-activating factor and leukocyte adherence and modulates adhesion molecule 1 expression. It was also reported that this E_1 analogue induces glycosaminoglycans production, and in synergy with colchicine, increases its anti-inflammatory and analgesic effect (57) (Table 1).

3.3.5. Chondroitin Sulphate

Chondroitin sulfate is a sulfated glycosaminoglycan (GAG) composed of a chain of alternating sugars (N-acetylgalactosamine and glucuronic acid). It is principally used as IC therapy because it is physiologically present on the urothelium. The rationale behind the use of chondroitin sulfate (CS), rather than pentosan polysulfate, heparin or the non-sulfated hyaluronan, is that chondroitin sulfate has only a slight effect on clotting cascades or it has a lower effector activity on coagulation pathway, with respect to heparin (58) or hyaluronan (59). Studies with CS have demonstrated that the urothelial barrier restoration effect of CS molecule is also associated with a suppression of inflammation. Thus, the intravesical instillation of chondroitin sulfate not only physically restores the urothelium barrier function to ions present in the urine concentrate, but also induces its *de-novo* production and reduces the related inflammatory effect resulting from IC pathology (60) (Table 1).

3.3.6. Hyaluronic Acid

Hyaluronic acid (HA) is a natural proteoglycan found in connective tissues as well as in mast cell secretory granules (61). HA was first clinically used in the treatment of articular joints in patients with osteoarthritis. It has been shown that abnormal levels of this molecule have been found in the urine of IC patients (62). These findings suggest that the damaged urothelial GAG layer releases HA into the urine, and thus its integrity could be restored by repeated HA instillation. The therapy involves weekly treatment for 4 weeks with 40 mg of HA, followed by monthly instillation for a further two months. Results have shown a significant but variable positive effect; nonetheless, there were no signs of toxicity or morbidity after the treatment (21). CD44 is a polymorphic transmembrane glycoprotein, which is primarily known as the hyaluronic acid receptor,

however it binds also to collagen, fibronectin, and ankyrin, thus it is involved in cell-cell adhesion and cell matrix cytoskeletal interactions (63). The fact that mast cells express CD44 on their surface additionally suggests that the hyaluronic acid action on IC patients may also be due to a significant inhibitory action on mast cells (64). Further studies confirmed that HA instillation into the bladder of experimental models of IC decreased the presence of mast cells, and reduced the presence of the intracellular adhesion molecule 1 (ICAM-1) and TNF-α more than in models treated with just heparin, thus, suggesting that ICAM-1 may play a role in the anti-inflammatory effect of HA (64). A recently published review indirectly compared the effects of three different therapies with mucosal surface protectors (such as high molecular weight hyaluronic acid, chondroitin sulfate and a combination of low molecular weight hyaluronic acid plus chondroitin sulfate). All the treatments induced detectable reduction in the perceived pain, and the repeated high molecular weight hyaluronic acid instillation, for 10 - 12 weeks, showed the maximum effectiveness; by contrast, low molecular weight hyaluronic acid preparation lacked any scientific evidence (65) (Table 1).

3.3.7. Liposomes

Biophysical studies confirm that liposomes are efficiently adsorbed by, or fuse or transfer lipids to the cell membrane, and can also be endocytosed by the cells, and thus, contribute to the urothelial membrane barrier effect (66). Additionally, it was also demonstrated that certain liposomal phospholipids are also able to modulate inflammation (67). Today, many liposomal compositions are available, or can be produced, that mimic the natural composition of the eukaryotic cell membranes (68). Even though it is well known that urothelial cells exhibit low endocytotic activity, many studies suggest that empty liposomes may help to restore urothelial barrier function (69). Recent clinical trials demonstrate that the intavesical injection of liposomes in IC patients, instilled once a week for 4 weeks 80mg in distilled water, is a safe and reliable method to regenerate the bladder epithelial barrier as well as to reduce the inflammation and pain symptoms (68) (Table 1).

3.3.8. Multimodal Therapies

IC has multifactorial causes correlated with many different etiological factors such as infections, autoimmunity, inflammatory processes, local neuronal dysfunction, bladder tissue abnormality, and toxins. Because of this multifactorial and recurrent nature it often requires different, as well as powerful treatments. In fact to achieve this aim, clinicians are nowadays adopting multimodal approaches by using different drugs; thus, different drugs, along with different mechanisms of action, would be able to act at different points during the manifestation of the disease.

A typical example of multimodal therapy confirmed that 6 and 9 months after the treatment, the concomitant treatment of IC patients with bladder hydrodistension and hyaluronic acid increased the duration of that treatment of patients treated with hydrodistension and heparin (70).

Then, it has been confirmed that the intravesical instillation of HA or heparin, both capable of restoring the integrity of the glycosaminoglycan layer, are improved by the addition of alkalinized lidocaine (AL), capable of reducing the pain in patients with severe interstitial cystitis (71). Continuing, another study also confirmed the positive effect of the concomitant treatment of IC patients using both sodium hyaluronate and chondroitin sulfate (72) (Table 1).

4. Conclusions

Even though positive results come from the concomitant use of different treatments, it is still desirable to test different and new drugs combinations. In addition, it is also necessary to develop new drugs and treatments that will embrace a therapy able to reduce bladder sensory nerve stimulation and thus pain, also inhibits neurogenic activation of mast cells, and provides urothelial cytoprotection, along with the anti-inflammatory activity. Consequently, clinicians and researchers are searching for new therapies.

Recently, human umbilical cord-blood-derived mesenchymal stem cells (UCB-MSCs) have been used in a preclinical study, investigating additionally the molecular mechanism. Results show that hUCB-MSCs injection significantly reduced the irregular and voiding interval stimuli, while in addition, they increased the epithelium thickening, and decreased the inflammatory response, and mast cell infiltration typically observed in the IC bladders. This study also demonstrated that hUCB-MSCs activate the Wnt signaling cascade interfering with the epidermal growth factor receptor activity (73).

The antiproliferative factor (APF) is present in the urine of approximately 95% of IC patients (as compared to approximately 9% of controls) and recent studies demonstrated that APF leads to urothelial cell proliferation blockade. The APF cell inhibitory mechanism is the result of this small molecule binding to the membrane resident palmitoylated CKAP4 receptors causing inhibition of cell proliferation and alteration of the expression of genes relevant to cell-to-cell permeability (E-cadherin, vimentin, and tight junction protein, ZO-1) (74). It was demonstrated that

siRNA-mediated knockdown of CKAP4 specifically abrogates APF's effects on cell proliferation, MMP2/p53 protein expression, and Akt/GSK3β/β-catenin phosphorylation on T24 bladder carcinoma cells (75). The Akt/GSK3β/β-catenin signaling importance for APF signal transduction was not only demonstrated in T24 cells (HTB-4), but was also confirmed in an hTERT immortalized human bladder epithelial cell line, TRT-HU1 (75).

In consequence, it is thought that the development of an APF antagonist drug would be able to reduce APF activity through Akt/GSK3β/β-catenin signaling, permitting urothelial cell proliferation and differentiation, along with GAG regeneration and thus epithelial barrier effect restoration (76).

Another study suggested that, since uterus and the urinary bladder share the same embryological origin, they would have also common molecular networks; among those networks chorionic gonadotropin (hCG) was demonstrated to have a pleiotropic action on bladder epithelial cells. Moreover, it was also found that hCG receptor is overexpressed by the urothelial cells (77).

In addition to that, IC symptoms seem to improve during pregnancy and during infertility treatments with hCG thus, Rao CV et al. (2016) concluded that hCG may have a therapeutic value against IC, and suggested to use it in adjunction to common IC therapies (78).

There is another problem that the clinician wants the researcher to solve while treating IC by intravesical drug instillation. Such concern is related to the drug delivery method because the simple instillation of a drug into the bladder is not so efficient. Thus, intravesical drug delivery is a challenge that researchers are trying to overcome having in mind that the drug should rapidly adhere to the urothelium after instillation, should not interfere with the urine flow or with the normal bladder functionality and finally should be maintained in the bladder for at least several hours.

Scientists are currently trying to use nano-carriers with different form, as well as different formulations (such as lipids, synthetic polymers and biopolymers, proteins, metals, inorganic- and organometallic compounds). With this in mind, Barthelmes J. and colleagues (2011) developed a muco-adhesive polymer composed of chitosan-thioglycolic acid (TGA) to be used as nano-carrier during the intravesical drug delivery (79). Their results demonstrated that the polymer was a useful tool for local drug application in the urinary bladder since it increased the residence time of the drug into the bladder without interfering with the bladder functionality.

This confirms that biomaterials can improve the residence time of a drug into the bladder, and even if this is an accessory aspect it is extremely important in order to reach the final result. From this point of view, it is desirable that researchers will continue through this research line, as well as along with the development of new drugs.

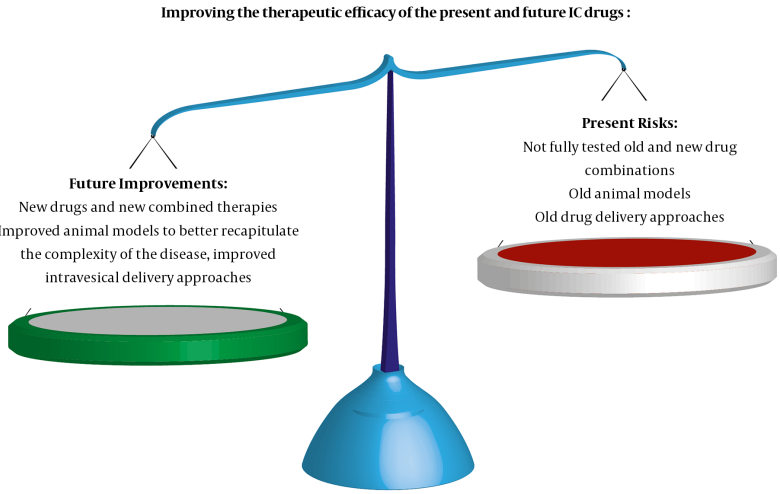
However, despite the results that researchers will obtain from the development of new drugs and new intravesical drug delivery systems, they are facing the needing for new or improved animal models.

In fact, despite CyP is probably the most commonly used IC inducing method, it has little etiologic basis in the clinical disease and there is also the possibility that systemic side effects may be the cause for a reduced clinical usefulness. In addition, the second most used IC inducing method, PS instillation, has also some limitations causing an extremely variable urothelial damage as well as inflammatory effects and also it is not able to induce bladder hypersensitivity and hyperactivity.

Continuing, some of the other already described IC inducing methods are instead preferentially used to cause inflammation and pain IC related symptoms, having little or scarce effects on GAGs and urothelial cells depletion. All this leads to errors and misinterpretations, which are unacceptable while testing the effect of a drug, in addition this will also cause useless an expensive extra experimental repetitions.

It is quite clear that there will be not just one animal model able to recapitulate all the IC symptomatology, thus also in my opinion it will be necessary to thoroughly test/study, compare and molecularly characterize new animal models obtained by combining the effects of different IC inducing molecules. This approach will be driven by the assumption that the new animal models should more widely mimic the symptom complexity and reflect the key characteristic of the disease, bladder/ pelvic pain. All these efforts will enhance the clinical and mechanistic significance of the models as well as the therapeutic efficacy of the future drugs, not only for those models with damage that is localized into the urothelium (Figure 5); however, to achieve this goal, much work remains.

Figure 5. Improving the Therapeutic Efficacy of the Present and Future IC Drugs



Present risks: Not fully tested old and new drug combinations, old animal models, old drug delivery approaches; future improvements: new drugs and new combined therapies improved animal models to better recapitulate the complexity of the disease, improved intravesical delivery approaches.

Footnotes

Authors' Contribution: Study concept and design, Ferro Federico acquired the data, drafted the manuscript and rewired manuscript for important intellectual content, critically.

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