
Intravesical Suplatast Tosilate (IPD-1151T) Inhibits Experimental Bladder Inflammation

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Purpose: Interstitial cystitis is a painful bladder disease characterized by urgency, frequency and variable inflammation but there is no curative therapy. Suplatast tosilate (IPD-1151T) is an immunoregulatory compound that decreases interstitial cystitis symptoms but to our knowledge its mechanism of action is unknown. We investigated the effect of intravesical IPD-1151T on mediator release from bladder explants in experimental cystitis.

Materials and Methods: A catheter was inserted into the bladder of female mice. After urine was emptied normal saline, carbachol (100 nM) or lipopolysaccharide (10 mg/ml) was introduced with or without 10-minute pretreatment with IPD-1151T. Urine was removed after 45 minutes for histamine and tumor necrosis factor- α assays. The bladder was removed after 4 hours, minced into 1 mm² pieces and cultured with or without triggers overnight for mediator release. The effect of IPD-1151T was also tested on rat skin vascular permeability as well as on purified rat peritoneal mast cells and human cord blood derived mast cells.

Results: Carbachol significantly increased histamine release in urine (61.3% in 8 preparations, $p < 0.05$) but not in explant medium. IPD-1151T inhibited this effect by 77%. Lipopolysaccharide induced a 350% urine histamine increase in 9 preparations ($p < 0.05$) and a 300% tumor necrosis factor- α increase in explant medium. IPD-1151T inhibited the lipopolysaccharide induced medium tumor necrosis factor- α increase by 95% in 5 preparations ($p < 0.05$). IPD-1151T did not inhibit rat skin vascular permeability or purified rat peritoneal mast cell activation by compound 48/80 or human cord blood derived mast cells by anti-IgE.

Conclusions: IPD-1151T inhibits bladder release of histamine and tumor necrosis factor- α through a mechanism that does not appear to involve direct mast cell inhibition. These findings may justify a beneficial effect of IPD-1151T in interstitial cystitis.

Key Words: bladder; cystitis, interstitial; histamine; inflammation; mast cells; tumor necrosis factor-alpha

Interstitial cystitis, a painful inflammatory bladder disease that occurs primarily in women, is characterized by bladder/pelvic pain, urgency, frequency and variable inflammation in the absence of urinary tract infection.¹ There is no curative treatment for IC.² A number of immunomodulators have been tested or proposed for IC.³ The 2 main theories of IC pathophysiology are some defect in the bladder protective glycosaminoglycan layer and an increased number of activated bladder mast cells,⁴ which is the only pathological finding that correlates with nocturia.⁵

Suplatast tosilate (IPD-1151T) is an immunomodulatory drug known to inhibit IgE antibody formation in BALB/c mice.⁶ It also suppresses IL-13 but not IL-4 production from human peripheral basophils.⁷ Use of IPD-1151T for 1 year improved IC symptoms and decreased blood eosinophils and IgE.⁸ IPD-1151T inhibits Th2 cytokine IL-4, IL-5 and IL-13 production in mice, thereby inhibiting eosinophil infiltration

into the murine airway, and the development of bronchial hyperresponsiveness in a murine model of asthma.⁹

We investigated the effect of intravesical IPD-1151T on the release of histamine and TNF- α in urine, and in the medium from bladder explants of female C57BL mice in which bladder inflammation was induced by intravesical administration of carbachol or LPS. The effect of IPD-1151T was also tested on purified RPMCs, mast cell dependent rat skin vascular permeability and hCBMCs.

MATERIALS AND METHODS

Materials

IPD-1151T powder (Taiho Pharmaceutical, Tokushima, Japan) was dissolved in sterile water freshly on the day of experiments and it was tested at the concentrations shown. Working dilutions were obtained in RPMI-1640, Locke's buffer or human Tyrode's buffer as necessary.

Animals

Female 10 to 12-week-old C57Bl/6 mice (Jackson Laboratories, Bar Harbor, Maine) were used. Animals were anesthetized with an intraperitoneal injection of a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg) in NS (0.9% NaCl). With its tip lubricated with glycerin a translucent 24

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gauge 3/4-inch catheter was introduced transurethraly into the bladder and advanced until the first drop of urine appeared in the hub (fig. 1). Pyrogen-free NS (0.15 ml) was instilled into bladder to wash and it was then removed together with any urine. NS or 10 μ M IPD-1151T (0.15 ml) was instilled into the bladder and allowed to remain for 30 minutes. The bladder was emptied and the solutions were collected for assay. Subsequently 0.15 ml NS as control, carbachol or LPS were instilled into the bladder and allowed to remain for 45 minutes. The syringe was kept on the catheter during this time to prevent leakage. Subsequently urine and the instilled solution were removed and stored at -80°C for mediator assays.

After the mice recovered from anesthesia they were allowed to remain in the cages for 4 hours and they were then sacrificed by cervical dislocation. Each bladder was removed, placed in chilled RPMI 1640 medium (Gibco, Grand Island, New York) and minced into 1 mm² pieces in a cold Petri dish on ice.¹⁰ Bladder explants were plated in separate wells in a 24-well tissue culture plate in 0.5 ml RPMI 1640 medium containing 10% fetal bovine serum, 100 U/ml penicillin/streptomycin and a protease inhibitor cocktail (Sigma®). Bladder explants were incubated overnight at 37°C in 5% CO₂. The next day medium was removed by aspiration and stored at -80°C for mediator assays. The remaining tissue was blotted and weighed before being discarded. This Protocol 50-02 was approved by the Institutional Animal Care and Use Committee.

Histology

Bladder tissues were fixed in freezing medium (Triangle Biomedical Sciences, Durham, North Carolina) immediately after collection. Cryostat sections (8 μ m) were prepared, dried and stored at -80°C until staining with 0.1% toluidine blue (pH 2) for mast cells and leukocyte identification.

Rat Skin Vascular Permeability

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, Massachusetts) weighing approximately 300 to 350 gm were housed 3 per cage, provided with free access to food and water, and maintained on a 14:10-hour light/dark cycle. Each animal was handled 1 at a time to minimize any stress. Rats were anesthetized with a single intraperitoneal injection of 0.2 ml ketamine (100 mg/ml) and 0.25 ml xylazine (20 mg/ml). They were injected intravenously (0.6 ml)

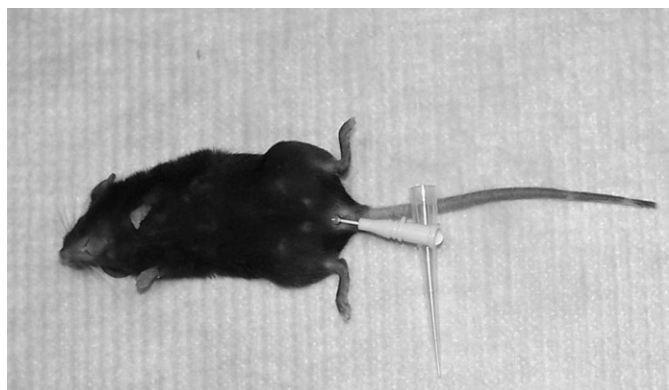


FIG. 1. Anesthetized female C57Bl/6 mice with catheter inserted intravesically.

via the tail vein with 1% Evan's blue 10 minutes after intradermal injection of the test substances. This approach decreased background extravasation. Pretreatment solutions of NS, IPD-1151T (10 μ M) and quercetin (10 μ M) were injected intradermally first using a tuberculin syringe. After 10 minutes the same area was injected with NS, CRH (10 μ M) or C48/80 (0.5 μ g/ml). Animals were sacrificed after 15 minutes by asphyxiation using CO₂ and decapitated. The abdominal skin was removed and photographed.¹¹

Stimulation of RPMCs

Rats were sacrificed by CO₂ asphyxiation and the peritoneal cavity was lavaged with Locke's buffer (pH 7.2). Cells were purified (95%) in 22.5% metrizamide in Locke's buffer, as described previously. Mast cells were pretreated with NS or IPD-1151T (10 μ M) before stimulation with bradykinin (10 mM).

Mediator Assay

Histamine and TNF- α were measured in urine collected after 45 minutes of instillation and in the tissue-free medium supernatant collected after overnight incubation of bladder explants using ELISA immunoassays (Beckman™ and R&D Systems, Minneapolis, Minnesota, respectively).

Stimulation of hCBMCs

hCBMCs were cultured as previously reported.¹² The hCBMCs were first sensitized with IgE for 48 hours and then pre-incubated with IPD-1151T (1 μ M) for 10 minutes before activating with anti-human IgE. The supernatant was collected and assayed for histamine by a LS-5B Luminescence Spectrometer (PerkinElmer, Norwalk, Connecticut), for tryptase by fluoroenzyme-immunoassay using the Unicap 100 automated instrument (Pharmacia and Upjohn, Uppsala, Sweden), and for IL-8 by ELISA (R&D Systems).

Statistical Analysis

Results are presented as the mean \pm SD. Results were compared using the paired Student t test or by nonparametric analysis using the Mann-Whitney U test with significance considered at $p < 0.05$.

RESULTS

We first used intravesical carbachol (10 μ M) to mimic the urgency and frequency experienced by patients with IC. Carbachol increased total urine histamine by 61.3% in 8 preparations compared to controls ($p < 0.05$). Intravesical administration of carbachol, LPS or saline did not induce the release of detectable levels of TNF- α in mouse urine. Pretreatment with IPD-1151T (10 μ M) in 5 preparations inhibited the carbachol induced histamine increase by 77% ($p < 0.05$, fig. 2). Intravesical administration of NS (control) released 0.4 ng/mg histamine from bladder tissue and intravesical administration of carbachol also released 0.4 ng/mg histamine from bladder tissue in 3 preparations without any further increase. Neither carbachol nor NS released detectable levels of TNF- α from bladder explants in 3 preparations on ELISA (sensitivity less than 0.05 pg/ml).

We then examined the effect of bacterial polysaccharide LPS on the assumption that patients with IC may have subclinical bacterial colonization. LPS increased total urine

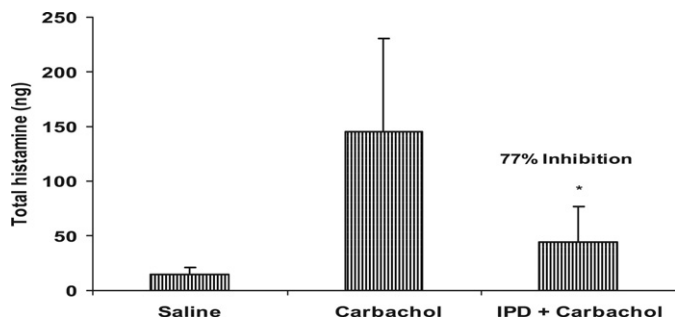


FIG. 2. Effect of IPD-1151T (IPD) (10 μ M) on intravesical carbachol induced urine histamine release in 8 and 5 preparations for carbachol, and IPD-1151T and carbachol, respectively. Asterisk indicates $p < 0.05$.

histamine release by 350% in 9 preparations compared to control ($p < 0.05$), while IPD-1151T could not inhibit this release (fig. 3, A). LPS also induced a 4-fold increase in TNF- α from bladder explants in 5 preparations compared to control ($p < 0.05$), while IPD-1151T pretreatment resulted in 95% inhibition (fig. 3, B). However, saline or LPS treatment induced the same amount of histamine release (1.6 ng/mg) from bladder explants in 3 preparations.

Bladder toluidine blue staining after intravesical carbachol showed individual changes in inflammatory infiltrates and IPD-1151T pretreatment did not have much of an observable effect (fig. 4, A and B). In contrast, intravesical LPS

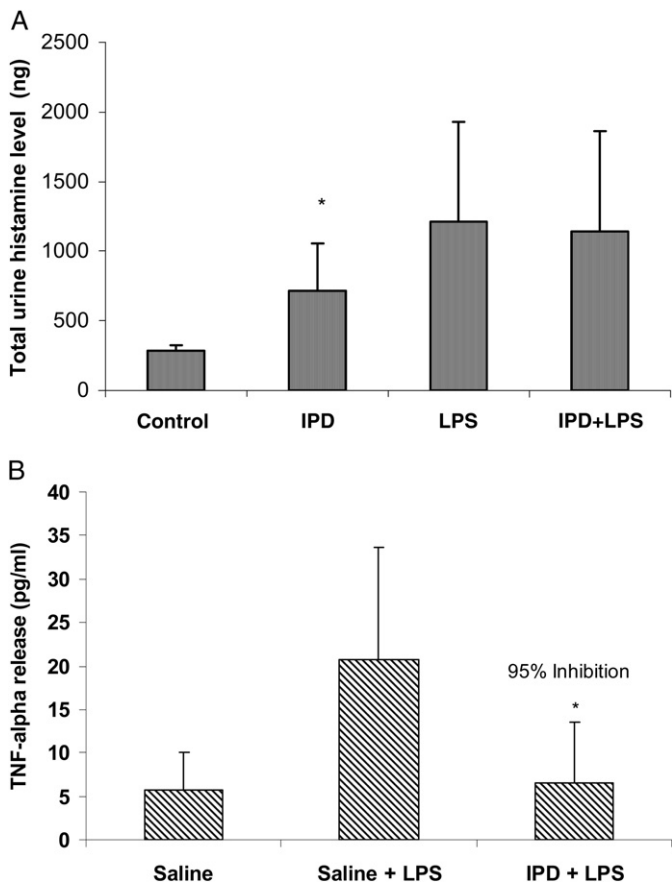


FIG. 3. Effect of IPD-1151T (IPD) (10 μ M) on intravesical LPS induced urine histamine release in 8 preparations for histamine and 5 for IPD-1151T (A), and LPS induced bladder explant TNF- α release in 5 preparations (B). Asterisk indicates $p < 0.05$.

induced leukocyte accumulation that was substantially decreased by IPD-1151T pretreatment and saline control (fig. 4, C to F). Intact or degranulated mast cells were identified (fig. 4, C to F).

In view of the fact that IPD-1151T inhibited carbachol induced urine histamine release we investigated if its effect was mediated through inhibition of mast cell secretion. IPD-1151T could not block mast cell dependent skin vascular permeability, while the flavonoid quercetin used as a control inhibited this effect (fig. 5).

We then investigated if IPD-1151T could inhibit histamine secretion from purified RPMCs. Bradykinin and IgE/anti-IgE significantly increased histamine release, although IPD-1151T did not inhibit bradykinin or IgE/anti-IgE induced histamine release (fig. 6). The same was true for hCBMCs stimulated with IgE/anti-IgE (fig. 7).

DISCUSSION

IC is a chronic, painful bladder disease characterized by urgency, frequency and variable inflammation. There are no reliable animal models of IC.¹ Cyclophosphamide induced cystitis¹³ and neurogenic cystitis¹⁴ have been used to study bladder cytokine expression. LPS has also been used to induce experimental cystitis.¹⁰ We used the stable acetylcholine analogue carbachol to mimic the urgency and frequency experienced by patients with IC, measured as total urine voided. We then used LPS to determine that there may have been some subclinical bacterial colonization of the

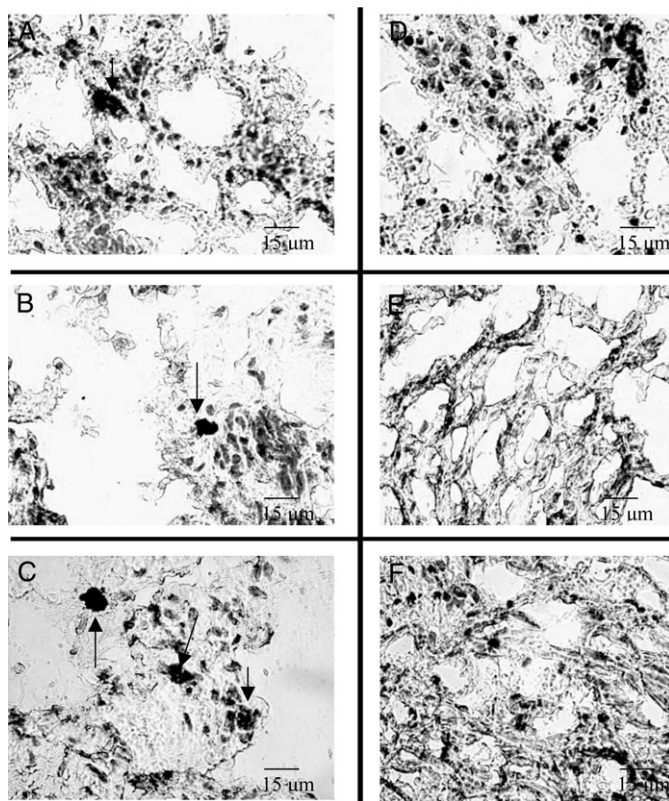


FIG. 4. Photomicrographs show bladder stained with toluidine blue after carbachol (A), IPD-1151T plus carbachol (B), LPS (C and D), IPD-1151T plus LPS (E) and saline (F) administration. LPS induced leukocyte accumulation (C and D) was substantially decreased by IPD-1151T pretreatment (E). Arrows indicate mast cells.

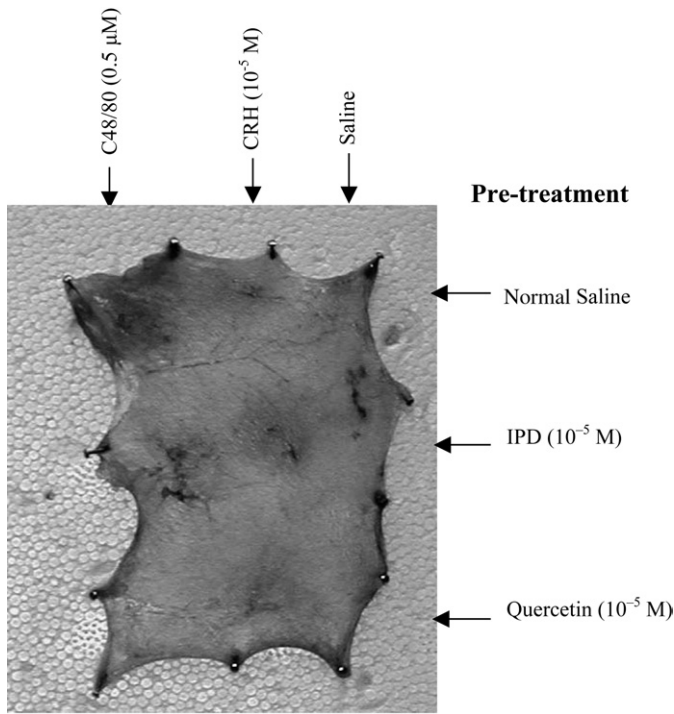


FIG. 5. Photograph reveals effect of IPD-1151T (IPD) (10 μM) and quercetin (10 μM) on vascular permeability. CRH and compound 48/80 induced substantial Evan's blue extravasation compared to NS, indicating mast cell activation. Quercetin but not IPD-1151T inhibited CRH and compound 48/80 induced Evan's blue extravasation.

bladder. To our knowledge this is the first report that IPD-1151T can inhibit bladder histamine release in response to carbachol and bladder explant TNF-α release in response to LPS. However, IPD-1151T could not inhibit LPS induced urine histamine release. Moreover, it did not have a direct inhibitory effect on skin and purified peritoneal mast cells or on human cultured mast cells.

The fact that IPD-1151T did not inhibit mast cells suggests that its inhibitory action on the release of carbachol induced histamine and LPS induced TNF-α release from mouse bladder is indirect and probably mediated through some other target. Such action could be on local nerve endings, which may release neuropeptides that could trigger mast cells. For instance, CRH could be released from dorsal root ganglia together with neurotensin¹⁵ and the 2 together could activate bladder mast cells. In fact, neurotensin receptor antagonist blocks stress induced bladder mast cell activation.¹⁶

IPD-1151T was reported to decrease IC symptoms.⁸ Neuroimmune interactions in the bladder were highlighted as having a key role in IC pathogenesis.¹⁷ Bladder mast cells are increased and activated in IC,⁴ and they are often found close to increased peptidergic nerve endings.¹⁷ More recently IPD-1151T was shown to decrease neuronal activation,¹⁸ suggesting that its action in bladder inflammation and possibly IC could involve the inhibition of sensory neuronal secretion of neuropeptide bladder mast cell triggers.

IPD-1151T could decrease IgE production,⁶ suppress hypersensitivity reactions, inhibit TH₂ cytokine production and suppress eosinophil infiltration in a murine model of asthma.⁹ IPD-1151T could also inhibit IL-13 release from human peripheral basophils.⁷

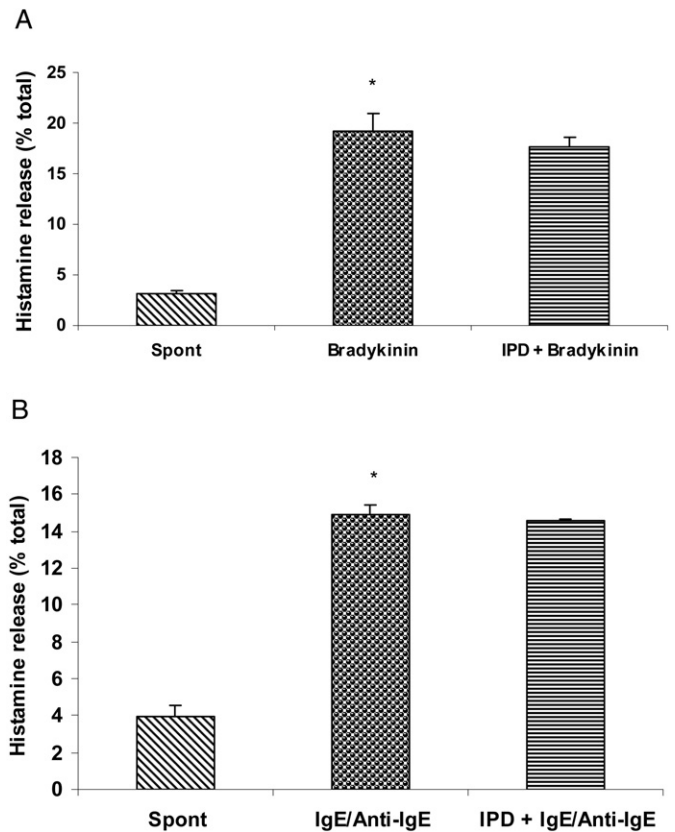


FIG. 6. Effect of IPD-1151T (IPD) on histamine release from RPMCs pre-incubated with IPD-1151T for 10 minutes before stimulation with bradykinin (10 mM) for 15 minutes (A) or IgE/anti-IgE for 30 minutes (B) in 3 preparations each. Histamine release was measured in supernatant fluid and pellet. Results are expressed as percent of total histamine in supernatant. Spont, spontaneous. Asterisk indicates p < 0.05 vs controls.

Mast cells are ubiquitous in the body and they are necessary for allergic reactions, during which they secrete vasoactive and nociceptive molecules as well as numerous cytokines.¹⁹ Mast cells are also involved in innate and acquired immunity as well as in neuroinflammatory conditions affected by stress.¹⁹ Mast cell derived TNF-α was involved in urothelial inflammation²⁰ and neurogenic cystitis was shown to be associated with TNF dependent trafficking

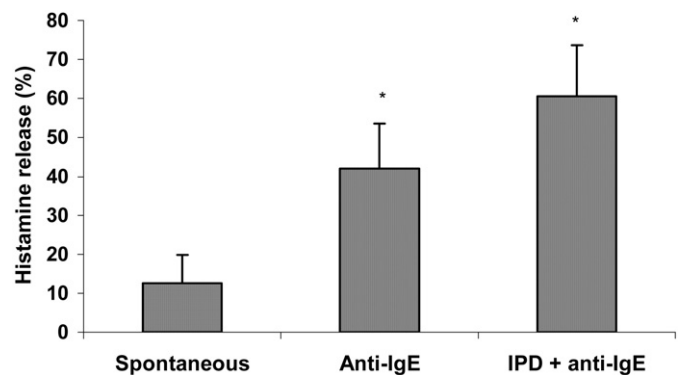


FIG. 7. Effect of IPD-1151T (IPD) (1 μM) on histamine release from hCBMCs in 3 preparations. IPD-1151T did not inhibit anti-IgE induced histamine release. Results are presented as percent of total histamine.

of mast cells from the detrusor to the lamina propria, where they increased 20-fold and were degranulated.¹⁴

CONCLUSIONS

IPD-1151T inhibits urine histamine and bladder explant release of TNF- α in an experimental model of bladder inflammation. These findings provide evidence of the immunomodulatory actions of IPD-1151T in the mouse bladder and provide further evidence for its consideration in IC treatment.³ The beneficial effect of agents affecting mast cell secretion in IC may be more apparent in patients with a history of allergies and/or bladder mastocytosis.³

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Abbreviations and Acronyms

CRH	=	corticotropin-releasing hormone
ELISA	=	enzyme-linked immunosorbent assay
hCBMCs	=	human umbilical cord blood derived cultured mast cells
IC	=	interstitial cystitis
IL	=	interleukin
LPS	=	lipopolysaccharide
NS	=	normal saline
RPMC	=	rat peritoneal mast cell
TNF- α	=	tumor necrosis factor- α
VEGF	=	vascular endothelial growth factor

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