Research Article

Chronic pain without persistent inflammation in an LL-37–induced interstitial cystitis/painful bladder syndrome model: Analgesic effects of a sulfated glycosaminoglycan ether

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Abstract

Background: Chronic bladder pain in interstitial cystitis often persists without overt inflammation, highlighting the need for physiologically relevant experimental models and effective non-opioid therapies. **Objective**: This study aims to evaluate the impact of persistent inflammation on bladder pain using an LL-37–induced murine model of interstitial cystitis/painful bladder syndrome (IC/PBS). We hypothesized that chronic pain could develop independently of ongoing inflammation and that a sulfated glycosaminoglycan ether (SAGE) compound could attenuate the pain and inflammation elicited by LL-37. **Methods**: Female C57BL/6 mouse bladders were instilled biweekly with 80 µM LL-37, a human antimicrobial peptide with immunomodulatory properties, for 1 h over four weeks. The analgesic efficacy of SAGE GM-0111, a sulfated hyaluronic acid derivative, was assessed by intravesical instillation immediately prior to LL-37 exposure. Pain responses were quantified using von Frey filaments (0.04–4.0 g). Inflammation and fibrosis were evaluated via myeloperoxidase and total collagen assays, gross examination, and histological analysis. **Results**: LL-37–treated animals exhibited significantly elevated pain responses (98.00 ± 0.42% positive response) compared to repeated saline controls (55.00 ± 3.80%; $p < 0.001$) and SAGE GM-0111–treated animals (54.17 ± 3.28%; $p < 0.01$). Despite pronounced hyperalgesia, no significant inflammation or fibrosis was detected by myeloperoxidase and collagen assays or histological evaluation. **Conclusion**: Repeated intravesical LL-37 administration induces a chronic pain phenotype that closely models human IC/PBS, wherein bladder pain occurs independently of overt inflammation. Furthermore, SAGE GM-0111 exerts significant analgesic effects in this model. This platform provides a valuable tool for elucidating mechanisms underlying bladder pain in IC/PBS, particularly in cases lacking evident inflammation or fibrosis.

Keywords: Cystitis, Bladder, Pain, Inflammation, Antimicrobial cationic peptides

1. Introduction

Interstitial cystitis/painful bladder syndrome (IC/PBS) is a debilitating chronic condition that predominantly affects women and substantially impacts quality of life. At present, it has limited effective treatment options.[1-6] Despite characteristic symptoms, many IC/PBS patients exhibit no significant cystoscopic findings.[7] The etiology of IC/PBS is poorly understood, and current theories include a defective glycosaminoglycan (GAG) layer that allows infiltration of inflammatory compounds from the urine into the urothelium, autoimmunity with chronic mast cell activation, and non-resolving neurogenic inflammation.[8-10]

Interstitial cystitis/painful bladder syndrome is a chronic

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disease, and, unfortunately, most research models are acute in duration, with the total study period from model creation to treatment outcome usually lasting less than seven days. The ability to repeatedly access the bladder is a challenge with most chemical-insult-based models, such as mustard oil, croton oil, turpentine, protamine sulfate, acetic acid, hydrochloric acid, xylene, or cyclophosphamide.[11] These chemical-induced cystitis approaches create profound inflammation, making repeated catheterization for intravesical access problematic, and their overall physiological relevance remains unclear. Therefore, generating a chronic model of IC/PBS-like bladder pain is necessary to advance our understanding of IC/PBS and to assess the effectiveness of new therapeutic interventions.

LL-37, a human antimicrobial peptide, is implicated in chronic inflammation in numerous organs, including the lungs, skin, bladder, bowel, and eyes.[12-15] It is naturally produced in the urinary tract and is elevated in patients with neurogenic bladder conditions, bladder outlet obstruction, or chronic urinary tract infections.[16-20] Our previous work has shown that LL-37 induces acute, dose-dependent local pain and inflammation in the bladder while mimicking key aspects observed in IC/PBS patients, including mast cell activation, inflammatory infiltrates, voiding dysfunction, and suprapubic pain.[21,22]

However, IC/PBS can also present with pain in the absence of histologic changes or distinct inflammatory features.[11,23] Based on observations of a dose-dependent pain and inflammatory response to LL-37,[21] we hypothesized that LL-37–induced cystitis could be used to create a chronic cystitis model mimicking human IC/PBS, in which bladder pain frequently occurs independently of inflammation.

Pain and discomfort are defining symptoms for IC/PBS and are primary issues that successful therapy must address with treatment approaches. Current therapeutic strategies for IC/PBS largely focus on reconstituting the bladder's GAG barrier or providing symptomatic pain relief.[24] Local anesthetics and neuromodulator agents—such as lidocaine or botulinum toxin—can transiently reduce discomfort but fail to promote urothelial repair or resolve an ongoing inflammatory process.[25-28] Pentosan polysulfate, a commonly prescribed therapeutic GAG, does not directly address inflammation or pain signaling.[29] These limitations highlight the need for therapeutics capable of simultaneously restoring urothelial integrity, suppressing inflammation, and alleviating pain.

Intravesical administration of semi-synthetic sulfated GAG ethers (SAGEs) may address the limitations of existing GAG therapies by combining urothelial barrier repair with robust anti-inflammatory and analgesic activity.[29] SAGEs exhibit a range of bioactive properties that extend beyond passive barrier restoration and include modulation of inflammatory signaling pathways.[29-31] Through these mechanisms, SAGEs have demonstrated the capacity to dampen inflammatory injury across diverse mucosal tissues and other diseases, including interstitial cystitis,[29,32] periodontal disease,[30,33] rosacea,[31] and smoke-induced lung inflammation.[34]

These findings suggest that SAGE-based therapies may be especially well suited for conditions such as IC/PBS, where epithelial dysfunction, persistent inflammatory signaling, and pain can occur independently of overt histopathology. Based on these properties, we selected SAGE GM-0111 for evaluation in the present LL-37–induced cystitis model to determine whether its combined barrier-protective and anti-inflammatory actions could mitigate bladder pain and dysfunction in a model that reflects key clinical features of IC/PBS. We hypothesized that SAGE GM-0111 is a potentially effective therapy for a chronic LL-37–induced cystitis model for IC/PBS that can help prevent both pain and inflammation.

2. Materials and methods

2.1. LL-37–induced cystitis model

LL-37 (single-letter amino acid sequence: LLGDFFRKSKEK IGKEFKRIVQRIKDFLRNLVPRTES) was synthesized by the DNA/Peptide Synthesis Core at the University of Utah and purified using preparative high-performance liquid chromatography. SAGE GM-0111 was obtained from Glycomira Therapeutics Inc. (United States of America [USA]). All experiments were approved and conducted in accordance with the University of Utah Animal Care and Use Committee (Protocol number: 1106010).

Animals were housed under standard conditions with a 12-hour light/dark cycle and had unrestricted access to food and water. Mice were randomly assigned to experimental groups and group-housed whenever feasible. All procedures were conducted during the light phase, either within the animal care facility or in a designated procedure room. Cages were bedded with Paperchip® (Shepherd Specialty Papers, USA), and environmental enrichment was provided using Enviropak Nestpak® containing envirodri® (Fibercore, USA). Animal health and welfare were monitored daily by a veterinarian or trained veterinary technician. The number of animals required for each study was determined based on the minimum number necessary to obtain adequate statistical power, and no animals were excluded.

Female C57BL/6 mice (8–10-week-old; n = 36) were randomly assigned to three groups: (i) 80 μM LL-37, (ii) 10 mg/mL SAGE GM-0111 followed by 80 μM LL-37, and (iii) sterile normal saline as a control (n = 12 per group). Following induction of anesthesia with isoflurane, mice underwent transurethral catheterization, through which a saline rinse was administered, followed by instillation of LL-37, as reported in previous studies.[22,29,35,36] The intravesical solution

was retained for a dwell period of 1 h. Each instillation was delivered slowly in a volume of 50 µL to avoid overdistension of the bladder and prevent vesicoureteral reflux. Catheters and syringes were maintained in position after delivery to ensure complete retention of the instilled fluid. For acute gross assessment, an additional subset of mice was euthanized 24 h after a single intravesical LL-37 instillation, and bladders were harvested for representative imaging. The chosen concentration of LL-37 was based on previous experiments to generate a significant pain response.[22]

2.2. Assessing bladder pain

To test whether LL-37–induced cystitis yielded chronic pain independent of inflammation, LL-37 was administered as described above twice weekly for four weeks, with euthanasia and tissue harvest on the fifth week post-initial instillation. For all treatment groups, baseline pain assessments were performed using von Frey filaments on all mice before their first catheterization. Post-instillation pain assessments were performed immediately before sacrifice, five days after the final instillation of LL-37.

Mice were positioned on a wire-mesh platform within clear enclosures to permit unobstructed observation. Following a minimum 10-min habituation period, mechanical allodynia in the suprapubic area was evaluated using von Frey filaments corresponding to 0.04, 0.16, 0.4, 1.0, and 4.0 g of stimulation, according to previously published procedures.[22,36] A sharp retraction of the abdomen, immediate licking or scratching of the stimulated area, or a jump was considered a positive pain response. Both positive and negative responses were recorded as data points. The investigator conducting the assessments was blinded to the treatment groups.

Pain assays were completed prior to animal sacrifice and tissue harvesting. For each stimulus intensity, nociceptive sensitivity was quantified as the percentage of positive responses observed across 10 applications per animal. For each animal, the change in response rate was calculated as the percentage of positive responses post-challenge minus the percentage of positive responses during baseline assessment. Mean changes in response rate at each stimulus intensity were then used for comparative analysis across the three experimental groups.

2.3. Tissue collection, gross imaging, histology, and tissue assays

Bladders were harvested by transecting the urethra and carefully removing the surrounding connective tissue. The organs were then hemisected along the median plane and imaged at 10× magnification using Olympus bx40 (Olympus, Japan). One-half section of bladder tissue was fixed in 4% paraformaldehyde (Sigma Aldrich, USA), and the remaining half was flash-frozen in liquid nitrogen and stored at −75 ± 5 °C until subsequent analysis. The fixed tissue was sectioned (5 µm), mounted on slides, and stained with hematoxylin and eosin (H&E) or Masson's Trichrome, then imaged at 200× magnification. Frozen tissues were processed, and myeloperoxidase (MPO) assays were performed using a murine MPO kit (catalog no. #HK210 Hycult®Biotech Inc., USA). Total collagen assays were performed using the BioVision Total Collagen Assay kit (catalog no. #26-K218-10 BioVision, USA). Tissue homogenates were centrifuged, and the resulting supernatant was processed according to the manufacturer's instructions.

2.4. Statistical analysis

One-way ANOVA was applied to the MPO and total collagen assay data. Data suitability for parametric testing was evaluated using the Shapiro–Wilk test for normality, and Grubbs' test was employed to detect and exclude any outliers. For the pain datasets, two-way ANOVA was applied, followed by Bonferroni-adjusted post hoc comparisons for analyses involving multiple groups. Statistical significance was defined as $p < 0.05$, with $p < 0.01$ and $p < 0.005$ defined as highly significant and very highly significant, respectively. All statistical analyses were conducted using GraphPad Prism 5.0 (GraphPad Software, USA).

3. Results

3.1. LL-37 elicits a chronic pain response

To better understand pain responses in mice exposed biweekly to LL-37, we evaluated suprapubic pain responses using mechanical stimulation (Figure 1A and 1B). At baseline, all mice demonstrated a typical logarithmic response pattern to stimuli ranging from 0.04 g to 4 g of stimulation (Figure 1C). Repeated catheterization and saline administration resulted in a minor, albeit not statistically significant, increase in the pain response rate compared with baseline values (Figure 1C and 1D). Animals that received 80 µM of LL-37 demonstrated a highly significant increase in pain response ($p < 0.01$) compared with baseline (Figure 1C). Sensitization from multiple weeks of LL-37 exposure created a substantial pain response that was inducible by suprapubic mechanical stimulation.

To evaluate the impact of SAGE GM-0111 in mice undergoing repeated bladder exposure to LL-37, we treated the animals with SAGE immediately prior to each LL-37 instillation over the full course of the study (Figure 1A). The degree of reduction was significant, bringing the pain response to near the level of mice that only received saline (Figure 1C and 1D). Differences between mice that received

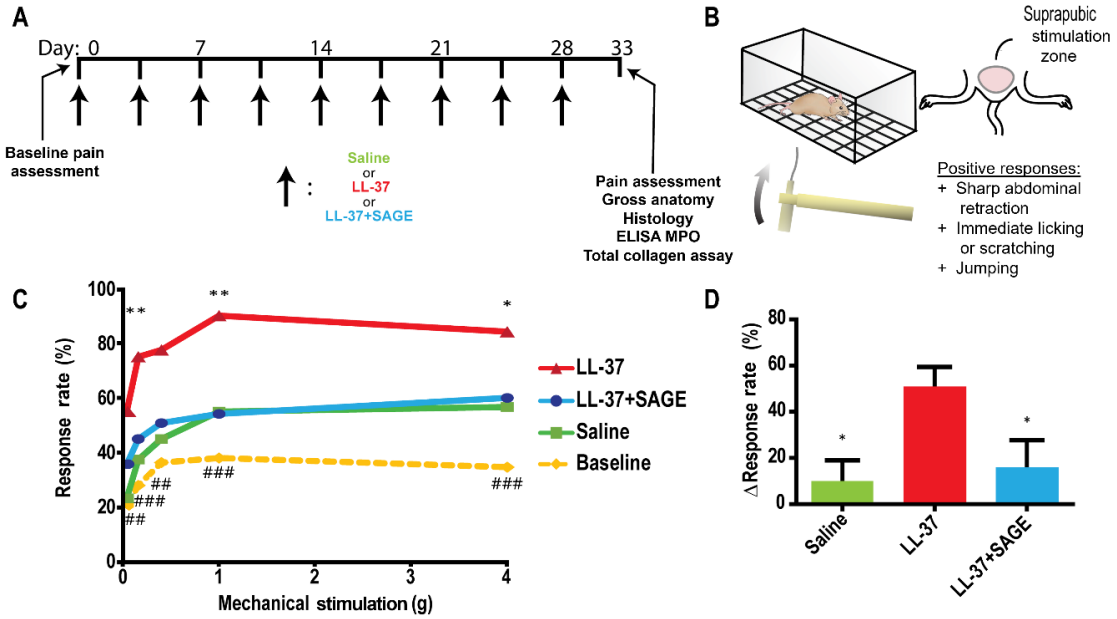

Figure 1. Pain response testing. (A) Schematic timeline of the study showing baseline assessment, biweekly intravesical administration, and treatment. (B) Schematic illustration of suprapubic assessment of sensitivity to mechanical stimulation used to evaluate pain. (C) Positive response rates of mice to filaments of varying stiffness (n = 12 per group; baseline values represent pooled baseline scores from all 36 mice). (D) Change in response rate relative to baseline (n = 12). Error bars represent the standard deviation. *$p < 0.05$ and **$p < 0.01$ indicate statistical significance between mice that received LL-37 alone and those that received LL-37 with SAGE pre-treatment. $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ indicate statistical significance for comparisons between baseline and LL-37–treated mice.
Abbreviations: ELISA: Enzyme-linked immunosorbent assay; MPO: Myeloperoxidase; SAGE: Sulfated glycosaminoglycan ether.

only saline and mice treated with SAGE GM-0111 followed by LL-37 were not statistically significant. Administration of SAGE GM-0111 reduced the mean pain response rate by 69% compared to untreated mice (Figure 1D). Overall, SAGE GM-0111 demonstrated a significant protective effect against pain sensitization induced by repeated LL-37 insult.

3.2. Inflammation assessment

To better understand how chronic LL-37 insult impacted inflammation, we performed gross anatomical observation, histology, and quantified MPO concentration via enzyme-linked immunosorbent assay (ELISA). LL-37 at 80 µM produced an acute inflammatory response within 24 h (Figure 2A). However, five days after the last LL-37 dose, gross anatomical examination of all mice in the LL-37–treated group demonstrated no visually apparent signs of inflammation, with no erythema, edema, or evidence of hemorrhage (Figure 2B). Microscopically, H&E histology confirmed the gross observations. Specifically, the histologic results demonstrated complete preservation of tissue architecture, with no disruption of the lamina propria, no polymorphonuclear leukocyte infiltrates, and no other cellular markers of inflammation (Figure 2B). MPO ELISA was used to quantify the presence and activity of neutrophils (Figure 2C). The saline control group demonstrated minimal tissue MPO levels (13.26 ± 1.52 ng/mL). The 80 µM LL-37 group and the SAGE pre-treatment group demonstrated MPO levels of 12.88 ± 0.80 ng/mL and 17.77 ± 10.72 ng/mL, respectively (Figure 2C). Quantified tissue MPO levels, an index of inflammation severity, did not differ between groups. After four weeks of LL-37 exposure, inflammation was not present at the observational time point, even without SAGE GM-0111, as measured by MPO.

3.3. Fibrosis

To assess the impact of LL-37 exposure on bladder fibrosis, we evaluated bladder tissues using trichrome staining and total collagen ELISA. Trichrome histology demonstrated no evidence of abnormal collagen deposition or fibrosis in the mucosa, submucosa, lamina propria, and bladder smooth muscle layer in any mice (Figure 3A). These histologic findings were further substantiated by total collagen assays. No group demonstrated any significant change in collagen deposition (Figure 3B). The saline control group exhibited 21.78 ± 6.55 µg/mL of collagen, which was statistically indistinguishable from the LL-37 group (19.73 ± 10.46 µg/mL) and the SAGE GM-0111 treated group (17.37 ± 6.79 µg/mL). This data, along with the gross anatomy and H&E findings, demonstrated no visual pathologic changes, scarring, or alterations in bladder tissue architecture in any of the groups.

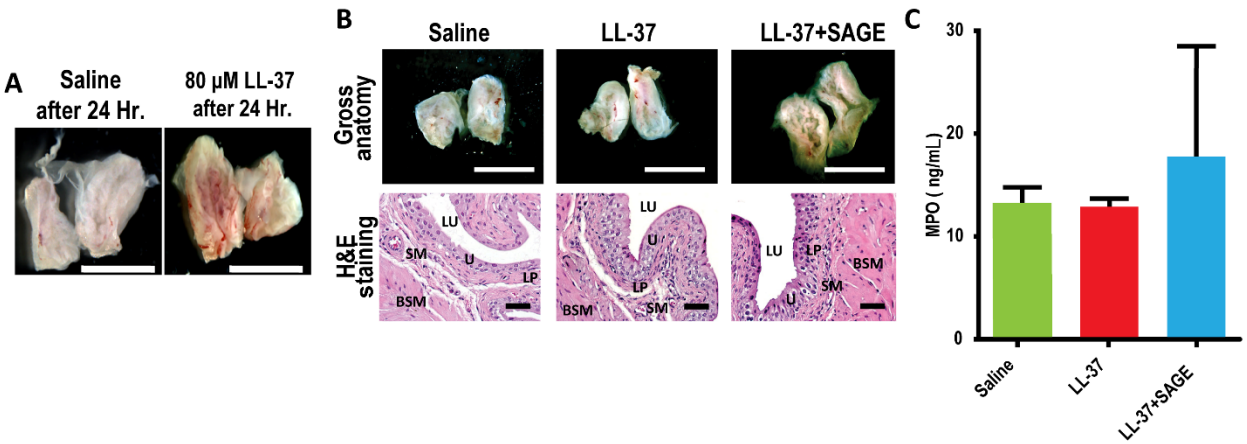

Figure 2. Bladder inflammation. (A) Representative gross anatomical images of the bladder 24 hours after LL-37 exposure. (B) Representative gross anatomical images and H&E-stained histological sections of bladders five days after four weeks of biweekly LL-37 exposure (gross images: scale bar = 5 mm, magnification = 10×; histology: scale bar = 50 µm, magnification = 200×). (C) MPO concentration in bladder tissue (n = 12 per group). Error bars represent the standard deviation.
Abbreviations: BSM: Bladder smooth muscle; H&E: Hematoxylin–eosin; LP: Lamina propria; LU: Lumen; MPO: Myeloperoxidase; SAGE: Sulfated glycosaminoglycan ether; SM: Submucosa; U: Urothelium.

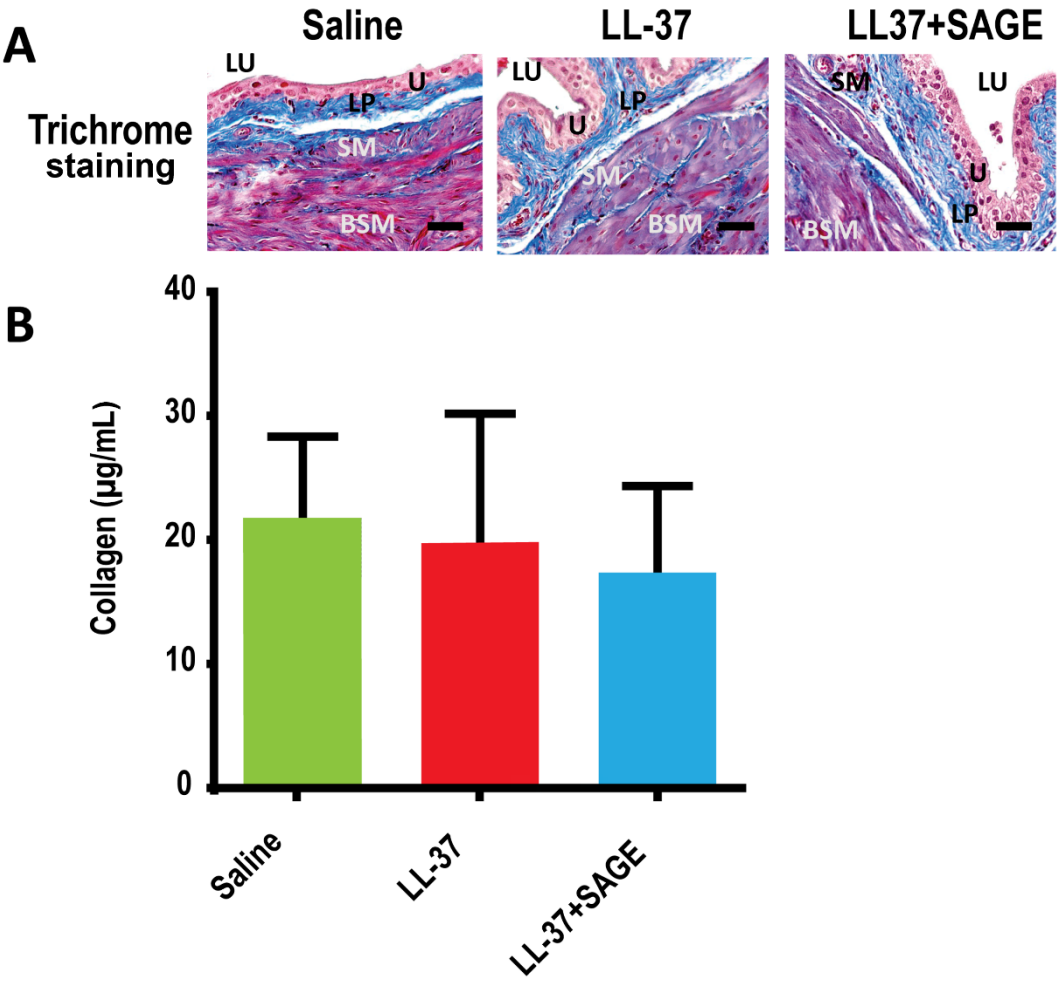

Figure 3. Assessment of bladder fibrosis. (A) Masson's trichrome-stained sections of the bladder wall (scale bar = 50 µm; magnification = 200×). (B) Bladder collagen content evaluated using a total collagen assay (n = 12 per group). Error bars represent the standard deviation.
Abbreviations: BSM: Bladder smooth muscle; LU: Lumen; LP: Lamina propria; SAGE: Sulfated glycosaminoglycan ether; SM: Submucosa; U: Urothelium.

4. Discussion

The findings reveal that repeated intravesical exposure to LL-37 produces a chronic bladder pain phenotype that persists in the absence of overt inflammation or fibrosis, and that prophylactic administration of SAGE GM-0111 significantly attenuates pain sensitization. This work demonstrates the feasibility of generating a chronic model of IC/PBS using repeated intravesical LL-37 exposure. Pain and discomfort are defining symptoms of IC/PBS and are primary issues that successful therapy must address through treatment approaches. We observed that pain was independent of inflammation in mice treated with LL-37, even after repeated insults. These findings provide valuable insights for longer-term studies using LL-37 to create more prolonged chronic models of IC/PBS, which may be useful for evaluating further potential therapeutic options.

We postulated that exposure to 80 μM LL-37 for four weeks would elicit significant cumulative pain without clear signs or evidence of an inflammatory response. Previous work in an acute LL-37–induced cystitis model showed that inflammation peaked after 24 h but was largely resolved by 72 h after insult.[22] In this present study, we observed the absence of ulcerative urothelial lesions, absence of tissue edema, and absence of increased MPO concentration, suggesting that inflammation resolved spontaneously even after repeated insults. However, pain responses were significantly elevated. Frequently, the degree of pain present and its impact on the quality of life in IC/PBS patients are independent of any observable inflammation or visual changes in bladder tissue on cystoscopy.[9,37-39] This is a key mystery in the etiology and pathophysiology of the majority of IC/PBS patients who present without inflammatory Hunner's lesions.[38,39] Future investigations using the LL-37–induced IC/PBS model may allow researchers to probe how nociception in the bladder becomes disconnected from inflammation and may enable deeper mechanistic insights into the causes that drive bladder pain. Further elucidating the mechanisms that drive bladder pain is essential for understanding why numerous patients experience substantial bladder pain without urothelial lesions or other obvious causes.

Limited insight into the pathophysiologic mechanisms underlying IC/PBS has made the condition challenging to model experimentally.[1,9,40,41] Many previously reported models for IC/PBS are based on chemically induced damage to bladder tissue, which does not accurately replicate the pathophysiological characteristics of the disease.[42] Unlike chemically induced cystitis models that rely on overt tissue injury, the LL-37–induced cystitis model is based on a natural physiologic compound found in distressed urinary systems, providing a physiologically relevant approach to modeling IC/PBS. This represents a distinct method from autoimmune-based models that use inoculations with tissue homogenates, bladder proteins, or genetically engineered mouse strains.[43,44] An LL-37 model could readily be deployed in genetically engineered mouse strains to investigate underlying mechanisms without extensive cross-breeding, which is both time-consuming and costly.

When discussing the applicability of our LL-37–induced cystitis model, this study further demonstrates how closely it mimics the human condition of IC/PBS. There is no perfect model that can completely replicate IC/PBS. However, we observed that bladder pain can exist independently of inflammation, demonstrating that the model can closely mimic IC/PBS. An independent study by Kim *et al*.[45] also used the LL-37 cystitis model to investigate mechanisms involving mast cells as a key cell type that perpetuates IC/PBS. Their study elucidated key molecular and cellular mechanisms of visceral pain and cross-organ sensitization involving the colon, demonstrating that mast cells and Mas-related G protein-coupled receptor B2 play a critical role in driving neural hypersensitivity, thereby contributing to pain, irritation, and physiologic dysfunction across multiple organ systems.

Although mechanistic pathways were not directly interrogated in the present study, prior work suggests that LL-37–induced pain may involve interleukin (IL)-33–mediated mast cell activation,[21] which may lead to downstream IL-31 signaling to sensory neurons. Several studies have demonstrated a potential IL-33/IL-31 inflammatory axis in different allergic and immune-mediated diseases, including atopic dermatitis, asthma, and other allergic disorders.[46,47] Mechanistic studies demonstrate that IL-31 can directly stimulate sensory neurons.[48-50] Although this potential mechanistic pathway has been described primarily in murine models or is active in other disease processes, it represents a potential area for further investigation and therapeutic intervention, including the use of an IL-31RA blockage (i.e., nemolizumab) in patients with IC/PBS if this pathway is relevant to the underlying etiology of IC/PBS. Although this inflammatory pathway was not studied in our chronic model, it represents a potential area for future studies to help elucidate the mechanisms underlying pain that occurs independently of inflammatory markers.

Non-opioid–based analgesic therapeutics for IC/PBS are greatly needed since a substantial number of IC/PBS patients report daily or persistent pain.[1,2,38] Our findings demonstrate that SAGE GM-0111 protects mice from LL-37–induced pain sensitization. In this study, a 69% reduction in pain response with 10 mg/mL of SAGE GM-0111 instilled into the bladder (~76.5 mg/kg) was greater than the 50% reduction observed with morphine (ID_{50} = 1.9 ± 1 mg/kg).[51] However, higher doses (~76.5 mg/kg) of SAGE were needed to achieve this effect. The protective mechanism of SAGE GM-0111 remains

uncertain and was not the primary objective of the present study. Prior mechanistic work with SAGE compounds has shown numerous potential modes of therapeutic action, including inhibition of human leukocyte elastase, the receptor for advanced glycation end-products, Toll-like receptors 2 and 4, nuclear factor κ-light-chain-enhancer of activated B cells, and P-selectin.[30,31] These effects may reduce extracellular ATP release, prevent urothelial cell apoptosis, decrease leukocyte elastase, inhibit immune cell infiltration into the bladder wall, and reduce mast cell infiltration and degranulation.[21,30,31,52] While the precise mechanism remains unclear, SAGE shows a statistically significant therapeutic effect on bladder pain, voiding, and inflammation.[29,32] Future studies are needed to elucidate the mechanisms responsible for the observed analgesic effects of SAGE GM-0111.

This study has several limitations that should be acknowledged; nevertheless, this model captures a clinically relevant pain phenotype and provides a reproducible platform for mechanistic and therapeutic studies. First, functional bladder outcomes such as urinary frequency, urgency, and dysuria were not directly assessed, which are common clinical findings in patients with IC/PBS. While behavioral pain responses provide insights into nociceptive sensitization, the absence of voiding analyses limits conclusions regarding functional bladder dysfunction. Independent LL-37–induced cystitis models[45] have assessed bladder function, demonstrating that LL-37–induced cystitis causes urinary urgency behavior. Second, although the model spanned a four-week period with biweekly instillations to approximate chronic disease, it can be argued that this duration may be insufficient to fully capture the progressive and relapsing nature of IC/PBS observed in patients. Several studies have demonstrated the induction of chronic inflammatory markers within four weeks,[53-55] which informed the design of our chronic model. Third, SAGE was administered prophylactically prior to inflammatory insult, which may not directly reflect clinical treatment paradigms in which therapy is initiated after symptom onset. SAGE GM-0111 has been shown to be effective not only in preventing but also in decreasing inflammation.[52] Although these limitations are notable, our model reproduces key aspects of IC/PBS, including persistent suprapubic pain in the absence of overt histopathologic inflammation, a feature commonly reported in patients. This work also establishes a reproducible platform for examining pain-driven disease mechanisms and evaluating candidate therapeutics targeting urothelial dysfunction and inflammatory signaling.

5. Conclusion

Repeated intravesical LL-37 administration induces a chronic cystitis model characterized by persistent bladder pain independent of inflammation, closely replicating features of human IC/PBS. SAGE GM-0111 demonstrates significant analgesic efficacy in this model, supporting its potential as a promising non-opioid therapeutic avenue. This model provides a reproducible and physiologically relevant platform for investigating IC/PBS pathophysiology and evaluating novel treatments.

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Conflict of interest

Siam Oottamasathien is an equity holder in GlycoMira Therapeutics. The other authors declare they have no competing interests or conflicts of interest to declare.

Author contributions

Conceptualization: Siam Oottamasathien, Wanjian Jia
Formal analysis: Mark Martin Jensen
Investigation: Austin Schults, Wanjian Jia
Methodology: Wanjian Jia, Austin Schults
Supervision: Siam Oottamasathien
Writing–original draft: Austin Schults
Writing–review & editing: Mark Martin Jensen

Ethics approval and consent to participate

The work presented in this manuscript was reviewed and approved prior to study initiation by the Institutional Animal Care and Use Committee of the University of Utah (Protocol number: 1106010). All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals. Animal care facilities at the University of Utah are accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC).

Consent for publication

Not applicable.

Data availability statement

Original data are stored on internal servers and are available from the corresponding author upon reasonable request.

Additional disclosure

Part of the findings presented in this manuscript was previously presented at the 2020 American Urological Association Annual Meeting (doi: 10.1097/JU.0000000000000827.06; titled Chronic LL-37 induced cystitis produces pain independent of inflammation and modified glycosaminoglycans are potent non-opioid analgesics). The event was originally for May 15-18th 2020 at Washington DC, however it was canceled due to the COVID 19 pandemic. Presentation was recorded and submitted virtually for judges, otherwise it was a published abstract in The Journal of Urology, Volume 203, Issue supplement 4, April 2020. Artificial intelligence-assisted technologies were used during manuscript revision to help streamline editing for clarity and cohesiveness, but were not used to generate data, perform analyses, or conduct core research activities.

References

1. Hanno PM, Erickson D, Moldwin R, Faraday MM. Diagnosis and Treatment of Interstitial Cystitis/Bladder Pain Syndrome: AUA Guideline Amendment. *J Urol*. 2015;193(5):1545-1553. doi: 10.1016/j.juro.2015.01.086
2. Koziol JA. Epidemiology of interstitial cystitis. *Urol Clin North Am*. 1994;21(1):7-20. doi: 10.1016/S0094-0143(21)00587-5
3. Konkle KS, Berry SH, Elliott MN, *et al*. Comparison of an interstitial cystitis/bladder pain syndrome clinical cohort with symptomatic community women from the RAND Interstitial Cystitis Epidemiology study. *J Urol*. 2012;187(2):508-512. doi: 10.1016/j.juro.2011.10.040
4. Gonzalez G, Vaculik K, Khalil C, *et al*. Experiences of Women With Interstitial Cystitis/Bladder Pain Syndrome: What Can We Learn From Women's Online Discussions? *J Urol*. 2023;209(1):208-215. doi: 10.1097/JU.0000000000002955
5. Li J, Yi X, Ai J. Broaden Horizons: The Advancement of Interstitial Cystitis/Bladder Pain Syndrome. *Int J Mol Sci*. 2022;23(23):14594. doi: 10.3390/ijms232314594
6. Davis NF, Brady CM, Creagh T. Interstitial cystitis/painful bladder syndrome: epidemiology, pathophysiology and evidence-based treatment options. *Eur J Obstet Gynecol Reprod Biol*. 2014;175:30-37. doi: 10.1016/j.ejogrb.2013.12.041
7. Morlacco A, Mancini M, Soligo M, *et al*. Relevance of the Endoscopic Evaluation in the Diagnosis of Bladder Pain Syndrome/Interstitial Cystitis. *Urology*. 2020;144:106-110. doi: 10.1016/j.urology.2020.06.032
8. Theoharides TC, Kempuraj D, Sant GR. Mast cell involvement in interstitial cystitis: a review of human and experimental evidence. *Urology*. 2001;57(6):47-55. doi: 10.1016/s0090-4295(01)01129-3
9. Killinger KA, Boura JA, Peters KM. Pain in interstitial cystitis/bladder pain syndrome: do characteristics differ in ulcerative and non-ulcerative subtypes? *Int Urogynecol J*. 2013;24(8):1295-1301. doi: 10.1007/s00192-012-2003-9
10. Inal-Gultekin G, Gormez Z, Mangir N. Defining Molecular Treatment Targets for Bladder Pain Syndrome/Interstitial Cystitis: Uncovering Adhesion Molecules. *Front Pharmacol*. 2022;13:780855. doi: 10.3389/fphar.2022.780855
11. Birder L, Andersson KE. Animal Modelling of Interstitial Cystitis/Bladder Pain Syndrome. *Int Neurourol J*. 2018;22(Suppl 1):S3-S9. doi: 10.5213/inj.1835062.531
12. Doss M, White MR, Tecle T, Hartshorn KL. Human defensins and LL-37 in mucosal immunity. *J Leukoc Biol*. 2010;87(1):79-92. doi: 10.1189/jlb.0609382
13. Pahar B, Madonna S, Das A, Albanesi C, Girolomoni G. Immunomodulatory Role of the Antimicrobial LL-37 Peptide in Autoimmune Diseases and Viral Infections. *Vaccines (Basel)*. 2020;8(3):517. doi: 10.3390/vaccines8030517
14. Sun L, Wang W, Xiao W, Yang H. The Roles of Cathelicidin LL-37 in Inflammatory Bowel Disease. *Inflamm Bowel Dis*. 2016;22(8):1986-1991. doi: 10.1097/MIB.0000000000000804
15. Frasca L, Lande R. Role of defensins and cathelicidin LL37 in auto-immune and auto-inflammatory diseases. *Curr Pharm Biotechnol*. 2012;13(10):1882-1897. doi: 10.2174/138920112802273155
16. Gupta S, Jackson AR, DaJusta DG, *et al*. Urinary antimicrobial peptides: Potential novel biomarkers of obstructive uropathy. *J Pediatr Urol*. 2018;14(3):238.e1-238.e6. doi: 10.1016/j.jpurol.2018.03.006
17. Gupta S, Preece J, Haynes A, Becknell B, Ching C. Differentiating Asymptomatic Bacteriuria From Urinary Tract Infection in the Pediatric Neurogenic Bladder Population: NGAL As a Promising Biomarker. *Top Spinal Cord Inj Rehabil*. 2019;25(3):214-221. doi: 10.1310/sci2503-214
18. Chromek M, Slamová Z, Bergman P, *et al*. The antimicrobial peptide cathelicidin protects the urinary tract against invasive bacterial infection. *Nat Med*. 2006;12(6):636-641. doi: 10.1038/nm1407
19. Nielsen KL, Dynesen P, Larsen P, Jakobsen L, Andersen PS, Frimodt-Møller N. Role of Urinary Cathelicidin LL-37 and Human β-Defensin 1 in Uncomplicated Escherichia coli Urinary Tract Infections. *Infect Immun*. 2014;82(4):1572-1578. doi: 10.1128/IAI.01393-13
20. Babikir IH, Abugroun EA, Bilal NE, Alghasham AA, Abdalla EE, Adam I. The impact of cathelicidin, the human antimicrobial peptide LL-37 in urinary tract infections. *BMC Infect Dis*. 2018;18(1):17. doi: 10.1186/s12879-017-2901-z
21. Martin Jensen M, Jia W, Schults AJ, Ye X, Prestwich GD, Oottamasathien S. IL-33 mast cell axis is central in LL-37 induced bladder inflammation and pain in a murine interstitial

cystitis model. *Cytokine*. 2018;110:420-427.
doi: 10.1016/j.cyto.2018.05.012
22. Jia W, Schults AJ, Jensen MM, *et al*. Bladder pain in an LL-37 interstitial cystitis and painful bladder syndrome model. *Am J Clin Exp Urol*. 2017;5(2):10-17.
23. Akiyama Y, Homma Y, Maeda D. Pathology and terminology of interstitial cystitis/bladder pain syndrome: A review. *Histol Histopathol*. 2019;34(1):25-32.
doi: 10.14670/HH-18-028
24. Buford K, Peters KM, Riedl C, *et al*. Global Consensus on Interstitial Cystitis/Bladder Pain Syndrome: An Update on Therapeutic Treatments. *Neurourol Urodyn*. 2026;45(1):46-53.
doi: 10.1002/nau.70106
25. Nickel JC, Jain P, Shore N, *et al*. Continuous Intravesical Lidocaine Treatment for Interstitial Cystitis/Bladder Pain Syndrome: Safety and Efficacy of a New Drug Delivery Device. *Sci Transl Med*. 2012;4(143).
doi: 10.1126/scitranslmed.3003804
26. Parsons CL, Housley T, Schmidt JD, Lebow D. Treatment of interstitial cystitis with intravesical heparin. *Br J Urol*. 1994;73(5):504-507.
doi: 10.1111/j.1464-410X.1994.tb07634.x
27. Welk BK, Teichman JMH. Dyspareunia Response in Patients with Interstitial Cystitis Treated with Intravesical Lidocaine, Bicarbonate, and Heparin. *Urology*. 2008;71(1):67-70.
doi: 10.1016/j.urology.2007.09.067
28. Nickel JC, Moldwin R, Lee S, Davis EL, Henry RA, Wyllie MG. Intravesical alkalinized lidocaine (PSD597) offers sustained relief from symptoms of interstitial cystitis and painful bladder syndrome. *BJU Int*. 2009;103(7):910-918.
doi: 10.1111/j.1464-410X.2008.08162.x
29. Lee WY, Savage JR, Zhang J, Jia W, Oottamasathien S, Prestwich GD. Prevention of anti-microbial peptide LL-37-induced apoptosis and ATP release in the urinary bladder by a modified glycosaminoglycan. *PLoS One*. 2013;8(10):e77854.
doi: 10.1371/journal.pone.0077854
30. Savage JR, Pulsipher A, Rao NV, *et al*. A Modified Glycosaminoglycan, GM-0111, Inhibits Molecular Signaling Involved in Periodontitis. *PLoS One*. 2016;11(6):e0157310.
doi: 10.1371/journal.pone.0157310
31. Zhang J, Xu X, Rao NV, *et al*. Novel sulfated polysaccharides disrupt cathelicidins, inhibit RAGE and reduce cutaneous inflammation in a mouse model of rosacea. *PLoS One*. 2011;6(2):e16658.
doi: 10.1371/journal.pone.0016658
32. Jensen MM, Jia W, Schults AJ, *et al*. Temperature-responsive silk-elastinlike protein polymer enhancement of intravesical drug delivery of a therapeutic glycosaminoglycan for treatment of interstitial cystitis/painful bladder syndrome. *Biomaterials*. 2019;217:119293.
doi: 10.1016/j.biomaterials.2019.119293
33. Gu Y, Raja V, Lee HM, Hong H, Prestwich G, Ryan ME. Therapeutic potential of a novel semi-synthetic-sulfated-polysaccharide to suppress inflammatory mediators in P. gingivalis LPS stimulated human monocytes/macrophages. *J Inflamm (Lond)*. 2021;18(1):26.
doi: 10.1186/s12950-021-00292-6
34. Hirschi-Budge KM, Tsai KYF, Curtis KL, *et al*. RAGE signaling during tobacco smoke-induced lung inflammation and potential therapeutic utility of SAGEs. *BMC Pulm Med*. 2022;22(1):160.
doi: 10.1186/s12890-022-01935-x
35. Oottamasathien S, Jia W, Roundy LM, *et al*. Physiological relevance of LL-37 induced bladder inflammation and mast cells. *J Urol*. 2013;190(4S):1596-1602.
doi: 10.1016/j.juro.2013.01.002
36. Rudick CN, Schaeffer AJ, Klumpp DJ. Pharmacologic attenuation of pelvic pain in a murine model of interstitial cystitis. *BMC Urol*. 2009;9:16.
doi: 10.1186/1471-2490-9-16
37. Doiron RC, Tolls V, Irvine-Bird K, Kelly KL, Nickel JC. Clinical Phenotyping Does Not Differentiate Hunner Lesion Subtype of Interstitial Cystitis/Bladder Pain Syndrome: A Relook at the Role of Cystoscopy. *J Urol*. 2016;196(4):1136-1140.
doi: 10.1016/j.juro.2016.04.067
38. Han E, Nguyen L, Sirls L, Peters K. Current best practice management of interstitial cystitis/bladder pain syndrome. *Ther Adv Urol*. 2018;10(7):197-211.
doi: 10.1177/1756287218761574
39. Van Moh F, Vetter J, Lai HH. Comparison of urologic and non-urologic presentation in interstitial cystitis/bladder pain syndrome patients with and without Hunner lesions. *Neurourol Urodyn*. 2018;37(8):2911-2918.
doi: 10.1002/nau.23812
40. Akiyama Y, Luo Y, Hanno PM, Maeda D, Homma Y. Interstitial cystitis/bladder pain syndrome: The evolving landscape, animal models and future perspectives. *Int J Urol*. 2020;27(6):491-503.
doi: 10.1111/iju.14229
41. Kwon WA. Animal Model of Interstitial Cystitis/Bladder Pain Syndrome. *Int Neurourol J*. 2018;22(Suppl 1):S1-S2.
doi: 10.5213/inj.1820edi.001
42. Song PH, Chun SY, Chung JW, *et al*. Comparison of 5 Different Rat Models to Establish a Standard Animal Model for Research Into Interstitial Cystitis. *Int Neurourol J*. 2017;21(3):163-170.
doi: 10.5213/inj.1734898.449
43. Altuntas CZ, Daneshgari F, Sakalar C, *et al*. Autoimmunity to uroplakin II causes cystitis in mice: a novel model of interstitial cystitis. *Eur Urol*. 2012;61(1):193-200.
doi: 10.1016/j.eururo.2011.06.028
44. Lin Y, Liu G, Kavran M, *et al*. Lower urinary tract phenotype of experimental autoimmune cystitis in mouse: a potential animal model for interstitial cystitis. *BJU Int*. 2008;102(11):1724-1730.
doi: 10.1111/j.1464-410X.2008.07891.x
45. Kim B, Wang Z, Meng X, *et al.* An interorgan neuroimmune circuit promotes visceral hypersensitivity. *Res Sq.* Preprint posted online March 17, 2025.
doi: 10.21203/rs.3.rs-6221928/v1
46. Murdaca G, Greco M, Tonacci A, *et al*. IL-33/IL-31 Axis in Immune-Mediated and Allergic Diseases. *Int J Mol Sci*. 2019;20(23):5856.

doi: 10.3390/ijms20235856
47. Di Salvo E, Ventura-Spagnolo E, Casciaro M, Navarra M, Gangemi S. IL-33/IL-31 Axis: A Potential Inflammatory Pathway. *Mediators Inflamm*. 2018;2018:1-8. doi: 10.1155/2018/3858032
48. Kim BS, Artis D. The sensory neuroimmune frontier. *Immunity*. 2025;58(5):1033-1039. doi: 10.1016/j.immuni.2025.03.018
49. Sonkoly E, Muller A, Lauerma AI, *et al*. IL-31: A new link between T cells and pruritus in atopic skin inflammation. *J. Allergy Clin. Immunol*. 2006;117(2):411-417. doi: 10.1016/j.jaci.2005.10.033
50. Cevikbas F, Wang X, Akiyama T, *et al*. A sensory neuron–expressed IL-31 receptor mediates T helper cell–dependent itch: Involvement of TRPV1 and TRPA1. *J. Allergy Clin. Immunol*. 2014;133(2):448-460.e7. doi: 10.1016/j.jaci.2013.10.048
51. Laird JMA, Martinez-Caro L, Garcia-Nicas E, Cervero F. A new model of visceral pain and referred hyperalgesia in the mouse. *Pain*. 2001;92(3):335-342. doi: 10.1016/S0304-3959(01)00275-5
52. Oottamasathien S, Jia W, McCoard L, *et al*. A murine model of inflammatory bladder disease: cathelicidin peptide induced bladder inflammation and treatment with sulfated polysaccharides. *J Urol*. 2011;186(Suppl 4):1684-1692. doi: 10.1016/j.juro.2011.03.099
53. Hernandez-Baixauli J, Abasolo N, Palacios-Jordan H, *et al*. Imbalances in TCA, Short Fatty Acids and One-Carbon Metabolisms as Important Features of Homeostatic Disruption Evidenced by a Multi-Omics Integrative Approach of LPS-Induced Chronic Inflammation in Male Wistar Rats. *Int J Mol Sci*. 2022;23(5). doi: 10.3390/ijms23052563
54. Ranneh Y, Akim AM, Hamid HA, *et al*. Induction of Chronic Subclinical Systemic Inflammation in Sprague-Dawley Rats Stimulated by Intermittent Bolus Injection of Lipopolysaccharide. *Arch Immunol Ther Exp (Warsz)*. 2019;67(6):385-400. doi: 10.1007/s00005-019-00553-6
55. Germolec DR, Shipkowski KA, Frawley RP, Evans E. Markers of Inflammation. In: *Methods in Molecular Biology*. New York, NY, USA: Springer New York; 2018:57-79. doi: 10.1007/978-1-4939-8549-4_5