INTERSTITIAL cystitis is characterized by bothersome lower urinary tract symptoms, including increased urinary frequency, increased bladder sensation, bladder discomfort and pain, which often severely impair patient quality of life. Histopathology reveals a varying extent of mast cell proliferation and inflammatory infiltrates as well as scarring and fibrosis at the end stage of disease.[1] However, its exact etiology remains unclear, hindering the development of effective therapeutic interventions.

Basically, IC diagnosis is based on symptomatology, cystoscopy and the exclusion of other bladder diseases.[2] On cystoscopy IC can be divided into 2 subtypes, including classic IC with Hunner ulcers (ulcerative lesions) and nonclassic IC without ulcers. Classic IC is associated with older patient age, more severe symptoms and more evident inflammatory histological reactions.[3] Recent studies focused on biomarkers to diagnose IC and/or discriminate between the 2 types of IC.[4] Candidate biomarkers include NGF,[5] CXCL9[4] and UPK III-δ4, a splicing variant of UPK III.[6]

Emerging evidence suggests that the TRP family of channels is likely to have important roles in regulating urothelial sensory perception and overall bladder function. TRPA1, TRPM7 and 8, and TRPV1, 2 and 4 are expressed in the urothelium of different regions of the urogenital tract.[7,8] $TRPV1^{-/-}$ mice feature abnormal bladder function, characterized by a high frequency of low amplitude and nonvoiding bladder contractions,[9] while TRPV4 stimulation facilitates the micturition reflex by activating mechanosensitive, capsaicin insensitive C fibers in rats.[10] In addition, functional ASICs, which are members of the amiloride sensitive epithelial Na^+ channel family, are expressed in the bladder of rats and mice.[11,12] An alteration in ASIC receptor expression was reported in a rat cystitis model.[13]

However, there is little or no clinical information on the comprehensive expression patterns and possible functional roles of these TRP channels and ASICs in IC pathophysiology. To explore IC pathophysiology, we comprehensively analyzed the mRNA expression of molecules that may be related to inflammation and/or mechanosensing/thermosensing, such as the TRP family, ASIC1 receptor, NGF, CXCL9 and UPK3A, using qRT-PCR.

MATERIALS AND METHODS

Subjects

Patients with IC scheduled for hydrodistention or those with noninvasive bladder cancer undergoing transurethral resection were enrolled as controls. The IC diagnosis was based on clinical guidelines for IC and hypersensitive bladder syndrome.[2] Patients with symptomatic bladder cancer or carcinoma in situ were excluded from analysis.

At study enrollment symptom severity was assessed by the OSSI, OSPI and VAS (score 0 to 10). The study protocol was approved by our institutional review board and fully explained to patients before written informed consent was obtained.

Using a rigid 18Fr cystoscope with the patient under spinal anesthesia, bladder specimens were obtained before hydrodistention or tumor resection with cold cup biopsy forceps from 1) retrotrigonal portions in nonclassic IC, 2) nonulcerative retrotrigonal portions in classic IC, 3) ulcerative portions in classic IC and 4) retrotrigonal, noncancerous, apparently normal portions in bladder cancer. The nonIC bladder served as the control. Samples were placed immediately in ice-cold RNAlater and stored at −80C. Total RNA was extracted from bladder samples and reverse transcribed into cDNA with RT. mRNA expression levels of several TRP channels (TRPA1, TRPM2, 7 and 8, and TRPV1, 2 and 4), ASIC1, NGF, CXCL9 and UPK3A were compared among the 3 groups. mRNA levels are shown as the fold change in the average control tissue value.

Total RNA Isolation

Total RNA was extracted from bladder samples by homogenization in Sepasol®-RNA I isolation solution according to the manufacturer protocol. RNA integrity and purity were assessed by capillary electrophoresis on a 2100 Bioanalyzer (Agilent Technologies, Palo Alto, California) with an RNA 6000 Nano LabChip® Kit and by measuring absorbance at 260/280 nm (A_{260}/A_{280} ratio). Only RNA samples with RNA integrity numbers of 6.5 or greater were considered for further analysis.

RT and qRT-PCR for mRNA Expression

Total RNA was reverse transcribed using the PrimeScript™ 1st Strand cDNA Synthesis Kit with 1 μg RNA and 6 mer random primers according to manufacturer instructions. qRT-PCR was performed with SYBR® Premix ExTaq™ II on a LightCycler®. The reaction volume was 20 μl, which contained 2 μl of a ten-fold dilution of cDNA, 10 μl SYBR Premix ExTaq II, 0.8 μl forward and reverse primers (10 μM each), and 6.4 μl water. qRT-PCR conditions were initial denaturation at 95C for 30 seconds, 40 to 45 amplification cycles with denaturation at 95C for 5 seconds, and annealing and extension at 60C for 20 seconds. Each experiment was done 3 times and average values were used for analysis. The primers used for qRT-PCR were selected using the Perfect Real Time Primer Support System (Takara Bio, Shiga, Japan) (see supplementary Appendix, http://jurology.com/).

Statistical and Data Analysis

For each sample gene expression levels were normalized to GAPDH and calculated as the fold expression relative to the median control value. The gene expression level was analyzed with the Wilcoxon rank sum test. Its relation to symptom severity was determined using the Pearson product moment correlation coefficient r with $p < 0.05$ considered statistically significant. R, version 2.13.1 (R Foundation for Statistical Computing, Vienna, Austria) was used for all statistical analysis.

RESULTS

Enrolled in the study were 11 controls, 17 patients with nonclassic IC and 22 with classic IC (table 1). All bladder cancers were noninvasive and not associated with carcinoma in situ. Bladder cancer grade and stage was G1Ta in 6 cases, and G1T1, G1T2, G2Ta, G2T1 and G3T1 in 1 each. Biopsy sites were histologically confirmed to be free of cancer. All IC cases were compatible with National Institute of Diabetes and Digestive and Kidney Diseases criteria. Patients with classic IC were older than those with nonclassic IC (mean ± SD age 70.6 ± 10.6 vs 56.1 ± 18.5 years, $p = 0.003$) and they appeared to be more symptomatic. Mean OSSI and OSPI were 14.1 ± 4.1 and 12.4 ± 3.2 in classic IC cases, and 13.1 ± 3.2 and 11.5 ± 3.4, respectively, in nonclassic IC cases. The mean VAS was 7.55 ± 1.85 and 5.25 ± 2.77 for classic and nonclassic IC, respectively. Patients with IC had had no hydrodistention, received no botulinum toxin injection therapy and used no steroids within the last year. All patients had been on nonsteroidal anti-inflammatory drugs on demand for bladder pain or discomfort.

qRT-PCR results indicated a significant increase in TRPV2 and NGF expression in nonclassic IC tissue, while there were major changes in classic IC tissue (table 2, and figs. 1 and 2). Of the TRP channels TRPA1, TRPM2 and 8, and TRPV1 and 2 showed significantly increased mRNA expression in nonulcerative portions of classic IC compared with controls. In the same portions we noted a significant increase in ASIC1, NGF and CXCL9 mRNA expression, and a significant decrease in TRPV4 and UPK3A mRNA expression. Also, in the ulcerative portions of classic IC we found a significant increase in TRPM2, TRPV1 and 2, and CXCL9, and a significant decrease in TRPV4 and UPK3A.

As assessed by the OSSI and OSPI or VAS, symptom severity was significantly associated with the TRPM2 and TRPV2 expression level (coefficient 0.266 to 0.516). TRPV1, ASIC1, NGF and CXCL9 levels significantly correlated positively with 1 or 2 symptom measures, and the TRPV4 level significantly correlated inversely (table 3).

Table 2. *mRNA expression in interstitial cystitis bladder tissue*

	Nonclassic IC		Nonulcerative Classic IC		Ulcerative Classic IC	
Gene Symbol	Fold Change	p Value	Fold Change	p Value	Fold Change	p Value
TRPA1	1.18	0.611	2.20	0.014*	1.45	0.143
TRPM2	1.26	0.578	3.71	0.007†	3.74	0.012*
TRPM7	0.85	0.225	0.92	0.560	0.83	0.166
TRPM8	0.64	0.487	1.51	0.021*	1.57	0.053
TRPV1	1.40	0.059	2.03	0.000†	1.15	0.040*
TRPV2	1.39	0.037*	2.07	0.000†	2.22	0.000†
TRPV4	1.04	0.547	0.68	0.036*	0.68	0.029*
ASIC1	1.34	0.134	2.34	0.000†	1.22	0.097
NGF	3.41	0.002†	2.81	0.014*	1.52	0.375
CXCL9	1.33	0.711	7.55	0.000†	6.04	0.006†
UPK3A	1.04	1.000	0.04	0.000†	0.07	0.000†

*p <0.05 vs control.
†p <0.01 vs control.

DISCUSSION

We compared mRNA expression levels of 7 TRP channels and ASIC1 as well as NGF, CXCL9 and UPK3A in bladder biopsy specimens from controls, and patients with nonclassic and classic IC to explore IC pathophysiology.

NGF mRNA levels were increased in each type of IC, suggesting that increased NGF expression may be a common IC change. Although the 1.5-fold increase in ulcerative lesions was not statistically significant in our study, it is comparable to a previous observation.[14] NGF, which is produced by bladder smooth muscle and the urothelium,[15] was implicated in altered bladder sensory function and the development of referred hyperalgesia in response to bladder inflammation[5] as well as in pathological conditions, such as idiopathic detrusor overactivity, neurogenic bladder and inflammatory bladder disease.[16]

In addition to NGF, TRPV2 mRNA levels were slightly but significantly increased in each type of IC. A few reports have shown a possible role for TPRV2 in bladder function. TRPV2 mRNA was found in cultured urothelial cells, urothelial tissue, dissociated smooth muscle cells and deepithelialized bladder tissue.[17] Since it is a heat and stretch activated channel, the function of TRPV2 could be to detect bladder filling or nociceptive sensation.

Table 1. *Patient background*

	Bladder Ca Control	Nonclassic IC	Classic IC
No. male/female	8/3	6/11	2/20
Mean ± SD age at diagnosis (range)	69.7 ± 12.3 (48-82)	56.1 ± 18.5 (20–73)	70.6 ± 10.6 (36–83)
Median yrs symptom history (range)		3.5 (1.0–28.0)	4.0 (1.5–20.0)
Mean ± SD score:			
OSSI	2.73 ± 1.0	13.1 ± 3.15*	14.1 ± 4.06*
OSPI	2.73 ± 1.27	11.5 ± 3.37*	12.4 ± 3.24*
VAS	0.55 ± 0.69	5.25 ± 2.77*	7.55 ± 1.85*

*p <0.05 vs control.

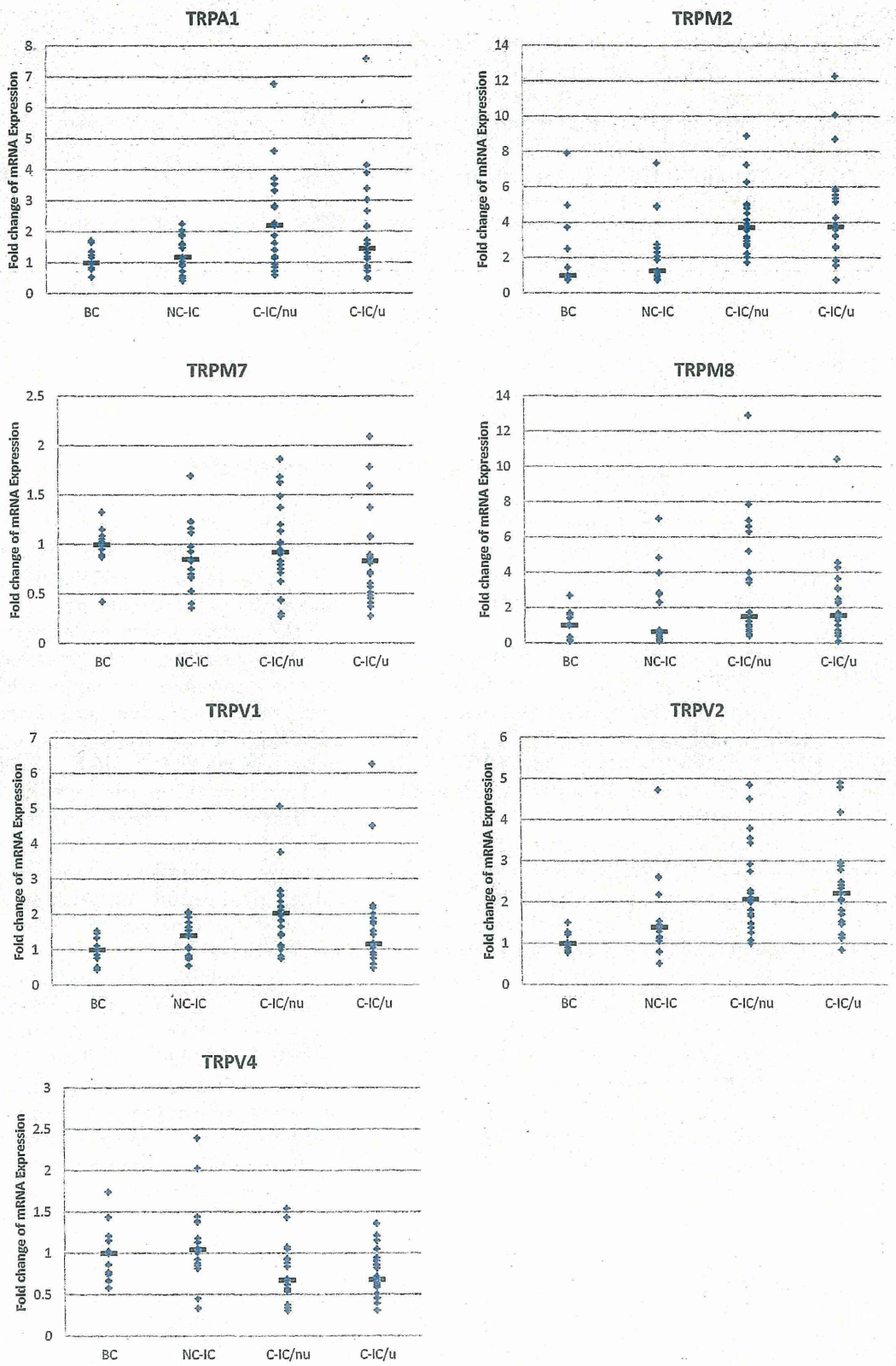

Figure 1. TRP channel mRNA expression in bladder tissue of each patient (dots), normalized to GAPDH and expressed as fold relative to median (horizontal lines) of controls. *BC*, bladder cancer. *NC-IC*, nonclassic IC. *C-IC/nu*, classic IC nonulcerative lesions. *C-IC/u*, classic IC ulcerative lesions.

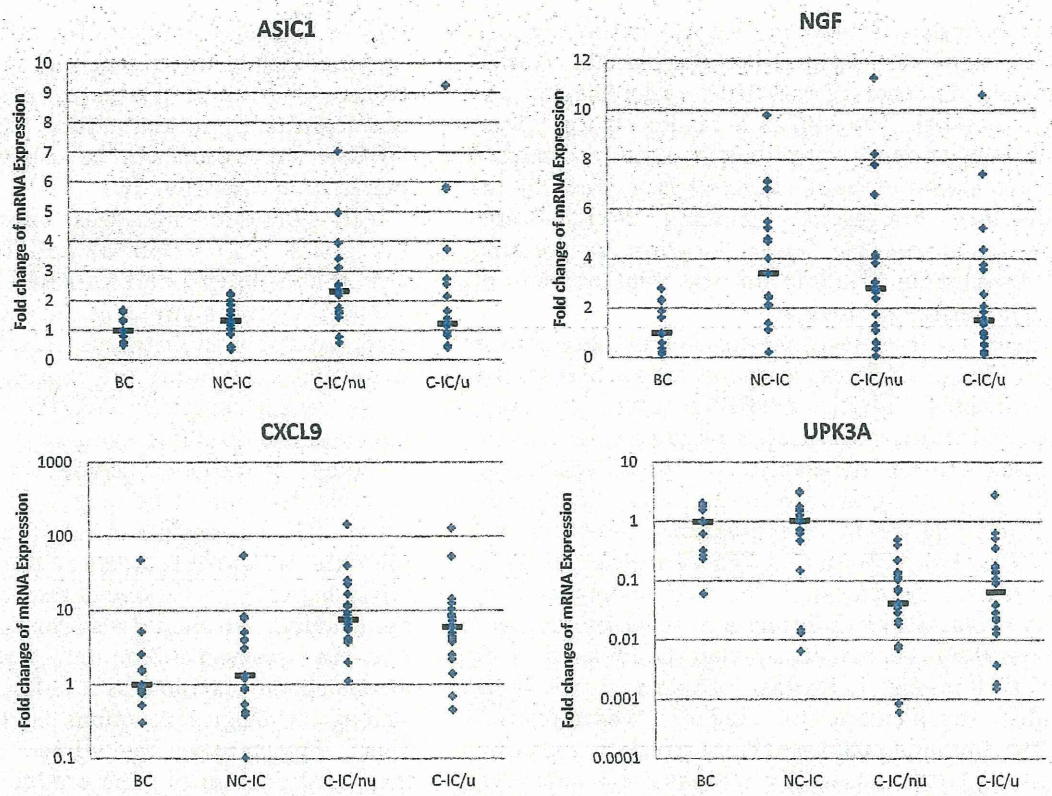

Figure 2. NGF, ASIC1, UPK3A and CXCL9 mRNA expression in bladder tissue, normalized to GAPDH and expressed as fold relative to median (horizontal lines) of controls. *BC,* bladder cancer. *NC-IC,* nonclassic IC. *C-IC/nu,* classic IC nonulcerative lesions. *C-IC/u,* classic IC ulcerative lesions.

We found the same expression pattern for TRPA1 and TRPM8. That is, each was increased only in nonulcerative lesions of classic IC. TRPA1 is found in sensory nerve fibers that innervate the bladder trigone[18] and pharmacological blockade of TRPA1 attenuated bladder overactivity in rats with spinal cord injury.[19] TRPM8 immunoreactive staining was observed in the urothelium and afferent nerve fibers in the human bladder and the number of positive nerve fibers increased in cases of idiopathic detrusor overactivity and painful bladder syndrome.[20] A selective TRPM8 channel inhibited the cold evoked pain response in mice.[21] These observations and our results indicate that increased expression of TRPA1 and TRPM8 mRNA may be involved in the nociceptive sensitization of classic IC.

Table 3. *Symptom severity correlated with RNA expression*

Gene Symbol	OSSI		OSPI		VAS	
	r	p Value	r	p Value	r	p Value
TRPA1	0.035	0.776	0.178	0.143	0.228	0.060
TRPM:						
2	0.331	0.005*	0.363	0.002*	0.316	0.008*
7	−0.032	0.791	0.068	0.582	0.003	0.983
8	0.017	0.888	0.156	0.202	0.130	0.288
TRPV:						
1	0.162	0.183	0.289	0.016†	0.185	0.128
2	0.266	0.027†	0.339	0.004*	0.516	0.000*
4	−0.291	0.015†	−0.273	0.023†	−0.233	0.055
ASIC1	0.194	0.109	0.308	0.010†	0.213	0.079
NGF	0.161	0.181	0.277	0.021†	0.172	0.145
CXCL9	0.282	0.019†	0.236	0.051†	0.002	0.988
UPK3A	0.006	0.966	−0.103	0.443	−0.061	0.607

*p <0.01.
†p <0.05.

TRPM2 mRNA was increased in each portion from patients with classic IC with the most pronounced increase of all of the TRP channels. To our knowledge this is the first study showing a relationship between increased TRPM2 expression and a bladder disorder. TRPM2 is a Ca^{2+} permeable, nonselective cation channel that is expressed highly in the brain and broadly in other tissues, and acts as a sensor for reactive oxygen species.[22] TRPM2 up-regulation in macrophages and microglia aggravates peripheral and spinal pronociceptive inflammatory responses, facilitating inflammatory and neuropathic pain.[23] These findings support the view that TRPM2 may be a primary factor in the proinflammatory neuropathic pain associated with classic IC.

Urinary pH strongly influences IC pain symptoms.[24] ASICs have emerged as key sensors of extracellular pH. Recent studies suggest that these channels have pivotal roles in the pathophysiology

of the initiation of inflammation and chronic pain.[25] ASICs were over expressed in the bladder urothelium and detrusor of rats with cyclophosphamide induced cystitis[13] as well as in bladder biopsy specimens from patients with bladder pain syndrome.[26] The expression of these channels is induced by inflammatory mediators, including NGF, brain-derived neurotrophic factor, 5-hydroxytryptamine and bradykinin, leading to the sensitization of bladder sensory pathways.

TRPV1 receptors are expressed on urothelial cells and C fibers,[9] and they respond to low pH. In the current study ASIC1 and TRPV1 expression levels were significantly increased in nonulcerative lesions of classic but not nonclassic IC. These results are partially inconsistent with those of a previous study indicating significant up-regulation of ASIC2a and ASIC3 but not ASIC1a and TRPV1 mRNA levels in bladder pain syndrome.[26] The discrepancy may be partly explained by differences in selection criteria.

Several lines of evidence reveal the importance of TRPV4 channels in bladder physiology and their possible involvement in bladder overactivity.[27] TRPV4 channels facilitate the micturition reflex by activating mechanosensitive, capsaicin insensitive C fibers in rats.[10] In our study we detected decreased TRPV4 mRNA expression levels in classic IC samples. The reason for the decrease is unclear but it may be clinically related to the abnormal bladder filling sensation in patients with IC.

The increase in CXCL9 levels was consistent with a previous report that CXCL9 together with other CXCR binding chemokines, such as CXCL10 and 11, is up-regulated in the bladder tissue of patients with ulcerative IC.[4] Serum levels of these substances are also increased.[28] A series of CXCR binding proteins attracts and stimulates monocytes/macrophages, and T, natural killer, mast and dendritic cells, leading to mucosal inflammation and tissue destruction.[28] Our finding that CXCL9 mRNA was increased in classic but not nonclassic IC suggests that this chemokine and the resultant inflammation are events specific to classic IC.

UPKs are barrier proteins expressed by urothelial cells and their expression is altered in IC.[29] We found remarkable down-regulation of UPK3A in classic IC, essentially the same as in a previous report.[6] Heavy suppression of UPK3A expression may be pathognomonic to IC, in that the increased permeability of the urothelium enhances the penetration of urinary substances into the bladder wall and results in inflammatory changes. Decreased UPK3A expression can be useful as a diagnostic marker for ulcerative IC.

All symptom measures correlated with the expression level of TRPM2 and TRPV2, and partly with that of TRPV1 and 4, ASIC1, NGF and CXCL9, supporting the significance of expressed genes in symptomatic manifestation. It is tempting to anticipate that modulating the expression or function of these genes, especially TRPM2 and TRPV2, may alleviate the disabling symptoms of IC.

Study limitations include the lack of healthy controls, the unmatched ages of patients with nonclassic IC, the possible effect of treatments used at the time of sample taking and the undefined pathophysiological interactions of increased expression of the mRNAs. We should also consider the possibility that the observed inflammatory reactions reflected physiological reactions as a defense mechanism, as well as pathological reactions caused by the disease. Most importantly, the direct evidence for an increased amount of gene products and their function, and the precise localization of the substances in bladder tissue should be explored. Further studies using patients with overactive bladder as a comparative control, or examining possible changes along with clinical progression or therapeutic responses are also warranted in the future.

CONCLUSIONS

This study demonstrated increased expression of the genes involved in pronociceptive inflammatory reactions, including TRPM2 for the first time to our knowledge, and TRPV1, TRPV2, TRPV4, ASIC1, NGF and CXCL9, in classic IC cases. Different expression patterns suggest distinct classic and nonclassic IC pathophysiologies. The genes or their products are potential candidates for use as biomarkers or novel therapy targets.

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Original Article: Clinical Investigation

On- and post-treatment symptom relief by repeated instillations of heparin and alkalized lidocaine in interstitial cystitis

Akira Nomiya,[1] Takashi Naruse,[2] Aya Niimi,[1] Hiroaki Nishimatsu,[1] Haruki Kume,[1] Yasuhiko Igawa[3] and Yukio Homma[1]

Departments of [1]Urology, [2]Community Health Nursing, and [3]Continence Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Abbreviations & Acronyms
AVV = average voided volume
DMSO = dimethyl sulfoxide
FVC = frequency volume chart
GAG = glycosaminoglycan
GRA = global response assessment
HBS = hypersensitive bladder syndrome
IC = interstitial cystitis
NGF = nerve growth factor
NIDDK = National Institute of Diabetes and Digestive and Kidney Diseases
NSAIDs = non-steroidal anti-inflammatory drugs
NUIC = non-ulcer type IC
OSSI/OSPI = O'Leary and Sant's symptom index and problem index
UF = urinary frequency
UIC = ulcer type interstitial cystitis
VAS = visual analog scale (for pain)

Correspondence: Akira Nomiya M.D., Department of Urology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. Email: nomiyaa-uro@h.u-tokyo.ac.jp

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Objectives: To examine outcomes of intravesical instillations of heparin and alkalized lidocaine in patients with interstitial cystitis.

Methods: Patients with interstitial cystitis refractory to conventional therapies were given a solution of 20 000 U heparin, 5 mL 4% lidocaine and 25 mL 7% sodium bicarbonate, intravesically, weekly for 12 weeks consecutively. The treatment was regarded as "effective", when patients rated "slightly improved" or "better" on a seven-graded scale of global response assessment. Other assessment measures included O'Leary and Sant's symptom index and problem index, visual analog scale for pain, and frequency volume chart variables.

Results: A total of 32 patients were enrolled in the study. The average age was 63.3 years. All participants had received hydrodistension 2.2 times on average, and fulfilled National Institute of Diabetes and Digestive and Kidney Diseases criteria. The therapy was effective in 60.0% of the patients at the fourth instillation, in 76.7% at the last instillation, and 90.0%, 46.7% and 16.7% at 1, 2 and 6 months after the last instillation, respectively. Most of other assessment measures improved significantly at the fourth instillation and further beyond until the end of therapy. On termination of therapy, the efficacy gradually diminished, yet mostly maintained statistical significance by 2 months post-instillation. No severe adverse events occurred.

Conclusions: A 12-week course of weekly intravesical instillations of heparin combined with alkalized lidocaine is safe and effective in relieving symptoms in interstitial cystitis patients. The effect of the treatment is maintained for 6 months. Further studies are required to optimize the number of instillations and maintenance intervals in order to maximize the therapeutic potential of simple or combined instillations in the management of interstitial cystitis.

Key words: heparin, interstitial cystitis, intravesical instillation, lidocaine.

Introduction

IC is characterized by a particular symptom complex with no identifiable causes.[1] The symptom complex, HBS, is defined as bladder hypersensitivity, usually associated with urinary frequency, with or without bladder pain.[2] No current treatments have a significant impact on symptoms over time, and as a result, patients are subject to numerous treatment modalities; from invasive to holistic therapies.[3–5]

One of the possible etiologies for IC is chronic and persistent deficiency of the GAG layer, which allows penetration of urine into the interstitial layer of the bladder, thereby causing inflammatory reactions.[6,7] Heparin, a family of sulfated polysaccharide resembling GAG, is believed to bind the defect of the GAG layer on bladder surface. According to the previous reports, intravesical heparin therapy is effective in approximately half of the patients; however, it cannot produce immediate relief of IC symptoms.[7] In contrast, immediate symptom relief can be attained by intravesical lidocaine therapy. The safety and

improved absorption of alkalized lidocaine was confirmed in IC patients, although the effects of alkalized lidocaine disappear within a few days.[8] Combination of heparin and alkalized lidocaine successfully attained immediate and sustained improvement; however, the patients were followed only for 2 weeks post-treatment, and backgrounds (i.e. age, sex, with or without ulcer) predictive of favorable response have not been explored.[9]

We tested the efficacy of 12-weekly intravesical instillations of a combination of heparin and alkalized lidocaine in patients with IC, and evaluated therapeutic outcomes up to 6 months after the last instillation. In addition, we examined the difference in therapeutic response according to their backgrounds.

Methods

Patients

Patients with IC refractory to conventional therapies were enrolled in the study. IC was diagnosed by three conditions: (i) lower urinary symptoms, such as urinary frequency, bladder hypersensitivity and/or bladder pain; (ii) bladder pathology proven endoscopically by Hunner's ulcer and/or mucosal bleeding after over-distension; and (iii) exclusion of confusable diseases, such as infection, malignancy or calculi of the urinary tract.[2] According to cystoscopic findings on hydrodistension, patients were categorized into two groups; UIC and NUIC. Symptoms were assessed by OSSI/OSPI. Scores six or more for both indices, despite present therapies (i.e. hydrodistension or oral drugs), were required for enrolment. At enrolment, patients' age at therapy, age at onset of IC, duration of IC symptoms, sex, number of hydrodistensions undergone before the therapy and distended bladder volume at the primary hydrodistension were recorded. Patients with an allergy to lidocaine, continuous macrohematuria, active urinary tract infection and hemorrhagic diathesis were excluded.

The protocol of the study was approved by our Institutional Review Board (#2205), and was fully explained to the patients before a written informed consent was obtained.

Therapeutic protocol

All patients were intravesically given a solution of 20 000 U heparin (Ajinomoto, Tokyo, Japan), 5 mL 4% lidocaine (Astrazeneca, Osaka, Japan) and 25 mL 7% sodium bicarbonate (Otsuka, Tokyo, Japan) weekly for 12 weeks consecutively at our outpatient clinic using an 8-Fr urethral catheter. The acidity of the solution was pH 7.5. At each treatment, patients voided before instillation, and were instructed to hold urine for 30 min after instillation. The solution was prepared under sterile conditions immediately before every instillation. Adverse events were monitored by urinalysis and interviewing patients.

Table 1 Patients' demographics

No. (male/female)	32 (3/29)
Mean age (years)	63.3 ± 13.8 (range 35–82)
Age at onset of IC (years)	60.0 ± 14.4 (range 25–74)
Duration of IC (years)	4.7 ± 3.5 (range 1–13)
Type of IC (UIC/NUIC)	17/15
Past treatment	
Hydrodistension	32 2.21 times on average (range 1–7)
Distended bladder volume at primary hydrodistension (mL)	570.0 ± 230.0 (range 200–1200)
DMSO instillation	10
Medicine	
Suplatast tosilate	18
Tricyclic antidepressant	11
NSAIDs	14
Others	6

Evaluation items

We used GRA as the primary outcome measure. Participants rated their symptoms on a seven-grade scale ranging from markedly worse (–3) to markedly improved (+3) compared with the baseline. Efficacy was classified as "effective" when participants reported slight (+1) to marked improvement (+3) on the GRA, otherwise efficacy was considered to be "not effective" or as "symptom recurrence" if it was during the follow-up period.[10]

Other assessments included OSSI/OSPI, VAS for pain and FVC variables. The efficacy was evaluated after the first, fourth and 12th instillations, and 1, 2 and 6 months after the last instillation. Withdrawal from the study without completing the treatment course was counted as drop-out.

Statistical analysis

Therapeutic outcomes were compared with the baseline values. For its skewed distribution, signed Wilcoxon's rank sum test for paired samples was carried out to compare the values of average voided volume, daytime urinary frequency and nocturnal urinary frequency. For other variables, Wilcoxon's signed rank test was used. Patients' background factors associated with therapeutic efficacy at the fourth instillation and 2 months post-therapy were examined by χ^2-test and Fisher's exact test. $P < 0.05$ was considered significant. All calculations were carried out with SPSS, version 18.0 (SPSS, Chicago, IL, USA).

Results

A total of 32 participants (29 women and 3 men) were enrolled in the study (Table 1). The mean age was 63.3 years (range 35–82 years). All participants were compatible with the NIDDK criteria.[11] Of them, 17 were categorized as UIC, and 15 as NUIC. All patients had received hydrodistension

Table 2 Global therapeutic response ($n = 30$)

	During therapy			Post-therapy		
	Week 1	Week 4	Week 12	1 Month	2 Months	6 Months
Responders†	10	18	23	27	14	5
Non-responders‡	20	12	7	3	16	25
Response rate (%)	33.3	60.0	76.7	90.0	46.7	16.7

†GRA: +1, +2 or +3. ‡GRA: 0, −1, −2 or −3.

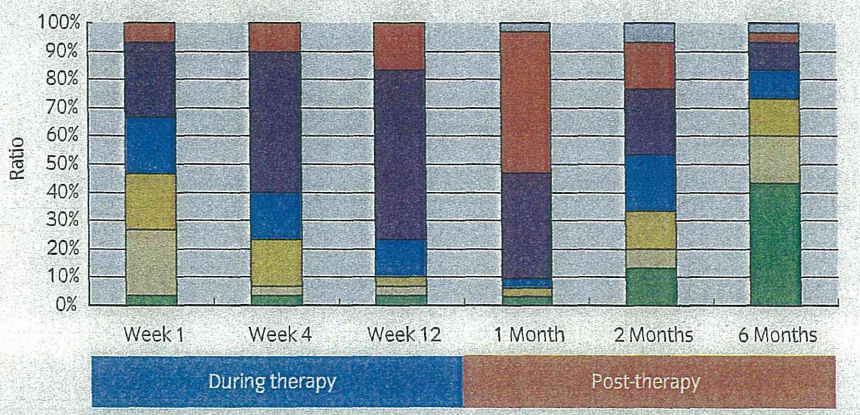

Fig. 1 Global response assessment for efficacy of heparin and alkalized lidocaine instillation. Patients with IC refractory to conventional therapies received a solution of 20 000 U heparin, 5 mL 4% lidocaine, and 25 mL 7% sodium bicarbonate intravesically weekly for 12 weeks consecutively. The patients were followed up at 1, 2 and 6 months post-instillation without further treatment. The efficacy was graded as "marked improved" (GRA +3), "moderately improved" (GRA +2), "slightly improved" (GRA+1), "no change" (GRA 0), "slightly worsened" (GRA −1), "moderately worsened" (GRA −2) or "marked worsened" (GRA −3). ■, GRA = +3; ■, GRA = +2; ■, GRA = +1; ■, GRA = 0; ■, GRA = −1; ■, GRA = −2; ■, GRA = −3.

at least once before instillation, with 2.2 times on average (range 1–7). Prior treatments included suplatast tosilate ($n = 18$), tricyclic antidepressant ($n = 11$), DMSO instillation ($n = 10$) and/or NSAIDs ($n = 14$). A total of 30 patients completed the treatment protocol and post-treatment follow up to 6 months, whereas two patients discontinued the therapy because of symptoms worsening at the fourth or sixth instillation.

According to GRA, responders gradually increased with advancement of the therapy (Table 2, Fig. 1); the response rate was 33.3% after the first instillation, 60.0% after the fourth and 76.7% after the 12th, and 90.0% 1 month after the last instillation. On the termination of instillation, the rate declined to 46.7% at 2 months and 16.7% at 6 months. Post-hoc analysis indicated ulcer type IC, onset age younger than 60 years and bladder volume at primary hydrodistension less than 500 mL as prognostic factors for better therapeutic response at the fourth instillation (Table 3); however, no factors were identified for efficacy at 2 months post-therapy. Other variables showed significant improvement during the therapy (Table 4). OSPI reached a significant level of improvement as early as at the fourth instillation ($P = 0.033$), and it was pronounced at the 12th instillation ($P < 0.001$). VAS for pain showed a significant reduction after the fourth instillation from the baseline ($P = 0.024$) and thereafter. Average voided volume significantly increased from the fourth therapy ($P = 0.029$). Urinary frequency decreased significantly at the fourth therapy for daytime frequency ($P = 0.003$) and for night-time frequency ($P = 0.001$). During post-therapy follow up, all the variables showed gradual deterioration with time; however, significant improvement lasted until 2 months after the termination of instillation. There was no significant difference at 6 months after the last instillation, except for nocturnal frequency, compared with the baseline.

As for side-effects of the therapy, no adverse events requiring additional intervention were observed. Two patients discontinued the therapy because of poor benefit. Minor side-effects included bladder pain ($n = 18$), gross hematuria ($n = 4$) and urinary tract infection ($n = 3$), all of which were self-limited. Gross hematuria was observed only on the day of instillation and not associated with systemic coagulation disorder (data not shown).

Additionally, 70% of patients reported slight bladder discomfort lasting for approximately 1 day every time after the administration, which also could be tolerated and decreased

Table 3 Univariate analysis of overall response factors at fourth therapy and 2 months post-treatment

	Univariate analysis					
	Fourth therapy			2 months post-treatment		
	OR	CI (95%)	*P*-value	OR	CI (95%)	*P*-value
Ulcer						
Ulcer/non-ulcer	8.250	1.154–59.003	0.039*	1.167	0.224–6.081	0.855
Sex						
Male/female	4.667	0.352–61.831	0.269	0.750	0.541–1.040	0.217
Age						
≥65 years/<65 years	0.500	0.087–2.886	0.657	1.250	0.233–6.715	0.795
Age at onset						
≥60 years/<60 years	0.121	0.017–0.867	0.039*	0.857	0.164–4.467	0.855
Duration of IC						
≥5 years/<5 years	1.500	0.266–8.449	0.685	1.167	0.224–8.081	0.855
Distended bladder volume						
≥500 mL/<500 mL	0.083	0.011–0.641	0.023*	1.250	0.233–6.715	0.795
Past hydrodistension						
≥2/once	0.686	0.119–3.963	0.673	2.4	0.444–12.980	0.414

*P-value <0.05.

Table 4 Therapeutic effects by symptom measures (mean ± SD, $n = 30$)

	Baseline	During therapy			Post-therapy		
		Week 1	Week 4	Week 12	1 Month	2 Months	6 Months
OSSI	13.4 ± 3.7	12.9 ± 3.4	10.9 ± 3.9	8.3 ± 3.6**	8.8 ± 4.0**	9.4 ± 4.4*	9.3 ± 5.3
OSPI	11.8 ± 4.2	11.5 ± 4.0	8.6 ± 4.2*	6.9 ± 3.3**	6.5 ± 3.6**	7.0 ± 3.9**	7.1 ± 4.5
VAS	5.8 ± 2.7	5.5 ± 2.4	3.4 ± 2.4**	3.5 ± 2.5*	3.3 ± 2.3**	3.8 ± 2.9*	3.5 ± 2.7
AVV1 (mL)	93.8 ± 65.5	108.2 ± 61.6	130.3 ± 61.8*	143.9 ± 72.9*	129.8 ± 66.4**	106.1 ± 72.4	118.6 ± 102.9
UF2 (day)	27.1 ± 36.6	19.3 ± 13.4**	14.4 ± 3.7**	13.4 ± 4.6**	14.6 ± 4.5**	15.1 ± 4.2*	15.0 ± 6.5
UF2 (night)	4.3 ± 2.7	3.6 ± 2.8**	2.7 ± 2.0**	2.3 ± 2.4*	2.3 ± 1.6**	2.1 ± 1.4*	2.0 ± 1.3*

*$P < 0.05$ versus baseline, **$P < 0.005$ versus baseline.

with continuation of the therapy. This discomfort was not related to therapeutic effect (data not shown).

Discussion

Intravesical therapy with a combination of heparin and alkalized lidocaine was first reported by Parsons.[9] The solution consisting of 40 000 U of heparin, 8 mL 2% lidocaine and 3 mL 8.4% sodium bicarbonate was given three times per week for 2 weeks. At the initial administration of the solution, 94% of patients (33 of 35 patients) reported immediate relief of both pain and urgency. However, patients were followed until 48 h after the last therapy, when 80% of them reported sustained relief of the symptoms. Another study by Welk used 10 000 U of heparin, 8 mL 2% lidocaine and 4 mL 8.4% of sodium bicarbonate for 23 female IC patients complaining of dyspareunia.[12] Patients were treated with the solution three times per week for 3 weeks. Three weeks after the therapy, 65% of patients reported a successful outcome of IC symptoms. Most of the efficacy parameters, including OSSI, OSPI, frequency, voided volume, Pelvic Pain Urgency Frequency score and Female Sexual Function Index pain domain score, showed significant improvement, supporting the effectiveness of the therapy. A double-blind, crossover, placebo-controlled trial showed that a single instillation of the solution can provide significant and immediate relief of IC symptoms up to 12 h.[13] These three studies demonstrated well the short-term efficacy, especially for pain, of intravesical therapy with a combination of heparin and alkalized lidocaine. However, they presented little data for outcomes post-administration.

Based on previous studies and the short-term efficacy of heparin instillation, we carried out the present study to assess the long-term outcomes of combined instillations, confirming the efficacy comparable with three previous studies. According to GRA, responders increased with advancement of the therapy; 33.3% after the first therapy, 60.0% at the fourth therapy and 76.7% at the 12th therapy. Once improved, there was no deterioration in efficacy during therapy. At the