INVESTIGATIVE AND CLINICAL UROLOGY

IC UROLOGY

Changes in uroplakin expression in the urothelium of patients with ulcerative interstitial cystitis/ bladder pain syndrome

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Purpose: We evaluated changes in the expression of uroplakin (UP) in the urothelium of patients with ulcerative interstitial cystitis/bladder pain syndrome (IC/BPS).

Materials and Methods: Bladder samples were collected from 19 patients with ulcerative IC/BPS who were treated with augmentation ileocystoplasty and from 5 control patients. Frequency-volume charts, the pain visual analogue scale (VAS), and the O'Leary-Sant interstitial cystitis symptom index (ICSI) and problem index (ICPI) were used to evaluate the patients' symptoms preoperatively. The expression levels of UP-Ib and UP-III in the urothelium were compared between the IC/BPS patients and control patients.

Results: Sixteen women and three men with IC/BPS were evaluated. Their values for preoperative mean voiding frequency, number of nocturia episodes, and functional bladder capacity as recorded in frequency-volume charts were 21.1±12.8, 5.9±4.2, and 151.1±62.7 mL, respectively. The mean pain VAS, ICSI, and ICPI scores were 8.4±1.3, 17.7±2.2, and 14.7±1.8, respectively. Immunofluorescence staining showed that UP-Ib and UP-III were localized in the urothelium. Upon Western blot analysis, the expression of UP-III was significantly increased in the IC/BPS group compared with the control group. However, expression of UP-Ib did not differ significantly between the IC/BPS and control groups.

Conclusions: UP-III was significantly upregulated in patients with ulcerative IC/BPS. UP-III is a potential biomarker for the diagnosis of ulcerative IC/BPS.

Keywords: Cystitis, interstitial; Urinary bladder; Uroplakins; Urothelium

INTRODUCTION

Interstitial cystitis/bladder pain syndrome (IC/BPS) is a chronic disease that considerably reduces quality of life as a result of uncontrolled storage symptoms and intense pelvic pain. Several hypotheses regarding the pathophysiology of IC/BPS have been suggested, but no consensus has been reached. In addition, IC/BPS is not easy to diagnose or treat. Urothelial dysfunction, which increases the permeability and decreases the protective function of the urothelium, is one potential cause of IC/BPS [1]. Various bladder tissue biomarkers related to urothelial dysfunction in IC/BPS have been explored, including uroplakin (UP) [2]. UP organizes the apical plaque on the surface of urothelial cells and has four

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subtypes in humans: UP-Ia, UP-Ib, UP-II, and UP-III. Each subunit of UP forms a heterodimer pair: UP-Ia combines with II and UP-Ib joins with III [3,4]. The UPs can affect the barrier function of the bladder urothelium, and the associated changes in the UPs may be related to IC/BPS.

Animal models can provide important clues about the pathophysiology of this disease. A comparison study of animal models of IC showed that injection of UP generates the most feasible animal model because it induces autoimmunity and increases the expression of inflammatory factors and fibrosis of the bladder tissue [5]. It is generally assumed that disruption of UPs destroys the integrity of the bladder's urothelial barrier and that entry of toxic materials induces IC/BPS. In contrast, one study reported significant upregulation of UP-III in the bladder tissue of patients with nonulcerative IC/BPS [6]. The relationship between changes in UP and IC/BPS is still unclear; however, so further research on UP conducted under more specific conditions is needed. We therefore evaluated the change in expression of UP-Ib and UP-III in the urothelium of patients with ulcerative IC/BPS.

MATERIALS AND METHODS

1. Study population

This study included 19 patients who were scheduled to undergo augmentation ileocystoplasty for IC/BPS at two centers. All patients had been diagnosed with ulcerative IC/BPS by characteristic clinical symptoms, exclusion of confusable diseases, and cystoscopic finding of a Hunner ulcer [7]. They were refractory to conventional management strategies using oral medication, hydrodistension, transurethral resection, or transurethral coagulation for Hunner ulcer. Five patients with muscle-invasive bladder cancer that required radical cystectomy and who had mild or no lower urinary tract symptoms and with a total International Prostate Symptom Score <8 were enrolled as the control group. Bladder tissue collection from the bladder after cystectomy for bladder cancer would not be ethically prohibited. In addition, bladder cancer patients with mild lower urinary tract symptoms could have normal bladder function, unlike patients with IC/BPS. All patients gave written informed consent before enrollment. Our study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the Bucheon St. Mary's Hospital Ethics Committee of The Catholic University of Korea (approval number: KC08TISS0309).

2. Study design

Frequency-volume charts, the pain visual analogue scale (VAS), and the O'Leary-Sant interstitial cystitis symptom index (ICSI) and problem index (ICPI) were used to assess the symptoms of patients with IC/BPS before their operations.

Bladder urothelium specimens were collected separately from ulcerative lesions in IC/BPS patients and separately from malignant lesions in the control group after cystectomy. The specimens were cut into sizes of approximately 1×1 cm each and were frozen in liquid nitrogen for preservation. The expression levels of UP-Ib and UP-III were investigated by using immunofluorescence staining and Western blotting. We compared the results between the ulcerative IC/BPS group and the control patients.

All data are described as the mean±standard deviation. The means were compared statistically using the Mann–Whitney U-test for all continuous variables. A p-value of <0.05 was considered to indicate a statistically significant difference.

3. Immunofluorescence staining

The specimens were embedded in an optimal cutting temperature compound and cut into 4-μm sections. The sections were warmed at room temperature for 30 minutes and then fixed in cold acetone for 2 minutes. The preparations were dehydrated, washed with phosphate-buffered saline (PBS), and then placed in a blocking solution for 60 minutes. The primary antibodies (anti-UP-Ib and anti-UP-III antibodies) were prepared, and immunolabeling of UP-Ib and UP-III was performed by incubation at 4°C after draining the blocking solution. The slides were washed three times with PBS for 5 minutes each time. Secondary antibodies (goat anti-rabbit immunoglobulin [Ig]G-fluorescein isothiocyanate antibody, 1:100) were then applied and the slides were incubated for 2 hours at room temperature in a dark chamber. After washing the slides three times with PBS for 5 minutes, an antifade reagent and a cover slip were applied. Staining was visualized using a fluorescence microscope, and digital images were captured.

4. Western blotting

We pulverized frozen tissues with a mortar and then lysed them at 4°C with radioimmunoprecipitation assay buffers. The lysates were centrifuged for 20 minutes at 13,000 rpm. The protein concentrations were measured with a Bio-Rad protein assay. Laemmli sample buffers were mixed with 50 μg of total protein and incubated for 5 minutes at 100°C before being applied to 10% sodium dodecyl sulfate-polyacrylamide gels. The proteins were moved to polyvinylidene fluoride membranes for 2 hours at 4°C with a semi-dry transblotting technique after electrophoresis. We blocked

Table 1. Clinical characteristics and preoperative symptom scores of IC/BPS patients and control patients

Characteristic	IC/BPS patients (n=19)	Control patients (n=5)
Age (y)	56.8±11.6 (43–73)	59.3±12.5 (51–70)
Sex		
Male	3	3
Female	16	2
Symptom duration (y)	4.2±2.2 (2–7)	N/A
Frequency-volume chart		
Frequency	21.1±12.8 (8–25)	4.8±0.8 (4–6)
Nocturia	5.9±4.2 (3–10)	0.6±0.5 (0–1)
Functional bladder capacity (mL)	151.1±62.7 (60–230)	342±40.9 (280–390)
Anesthetic bladder capacity (mL)	518.8±201.9 (250–920)	N/A
Pain visual analogue scale	8.4±1.3 (7–10)	N/A
O'Leary-Sant IC symptom index	17.7±2.2 (14–20)	N/A
O'Leary-Sant IC problem index	14.7±1.8 (12–16)	N/A

Values are presented as mean±standard deviation (range) or number only.
IC/BPS, interstitial cystitis/bladder pain syndrome; N/A, not applicable.

nonspecific binding sites by incubation in 1× Tris-Tween buffered saline containing 5% skim milk and 0.2% Tween 20 for 1 hour. The membranes were incubated overnight at 4°C with UP-Ib and UP-III antibodies. Western blot analysis was conducted with rabbit polyclonal antibodies against UP-Ib and UP-III. The membranes were incubated with rabbit IgG secondary antibody against HRP for 60 minutes at room temperature. The protein signals were detected with an enhanced chemiluminescence solution. Those membranes were also probed with a beta-actin monoclonal antibody followed by a mouse IgG secondary antibody, which were used as an internal loading control. Immunoreactive proteins were perceived, and scanned films were quantified with a gel documentation system.

RESULTS

The clinical characteristics and preoperative symptom scores of the IC/BPS patients and control patients are shown in Table 1. There was no significant difference between the IC/BPS patients and the control patients in terms of age. The IC/BPS patients had clinically severe symptoms (scores ≥12) and problems (scores ≥12) [8] and a smaller functional bladder capacity.

Immunofluorescence staining showed that UP-Ib and UP-III were localized in the urothelium (Fig. 1). In the Western blot analysis, the expression of UP-III was significantly increased in the IC/BPS group (0.962±0.201; range, 0.691–1.144) compared with the control group (0.516±0.072; range, 0.462–0.574) (p<0.001). However, UP-Ib expression was not significantly different between the IC/BPS group (0.373±0.083; range, 0.179–0.425) and the control group (0.450±0.065; range,

0.351–0.532) (p=0.082) (Fig. 2).

DISCUSSION

In this study, we identified changes in UP, particularly UP-III, in the urothelium from nonlesional areas in patients with a clinical diagnosis of ulcerative IC/BPS who were scheduled for augmentation ileocystoplasty. Plaques of UPs cover the apical surface of the urothelium and are known to prevent the penetration of substances in urine into the deeper layers of the urothelium. Two heterodimers, a UP-Ia/II pair in the inner subdomain and a UP-Ib/III pair in the outer subdomain, combine to form hexagonally arranged, 16-nm UP particles [9]. Although these two UP heterodimers are required to form the urothelial plaque, each subunit has a unique function. The outer subdomain, which is occupied by a UP-Ib/III pair, contains a transmembrane tunnel with both an extracellular opening and an intracellular opening. Although the inner subdomain also has a tunnel, this tunnel does not seem to traverse the entire subdomain [10]. Owing to these structural characteristics, the UP-Ib/III pair was thought to be more involved in the permeability barrier function of urothelial plaques than UP-Ia/II; therefore, we selected UP-Ib and UP-III for this study.

A previous study of UP-III knockout mice reported that a deficiency of UP-III induced a decreased number of apical urothelial plaques; thus, UP-III is likely a fundamental factor of urothelial plaques [11]. The ablation of UP-III in apical urothelial plaque is known to increase the permeability of the urothelium to water and urea [12]. In a different bladder function study that examined UP knockout mice, depletion of UP-III produced a significant increase in detrusor over-
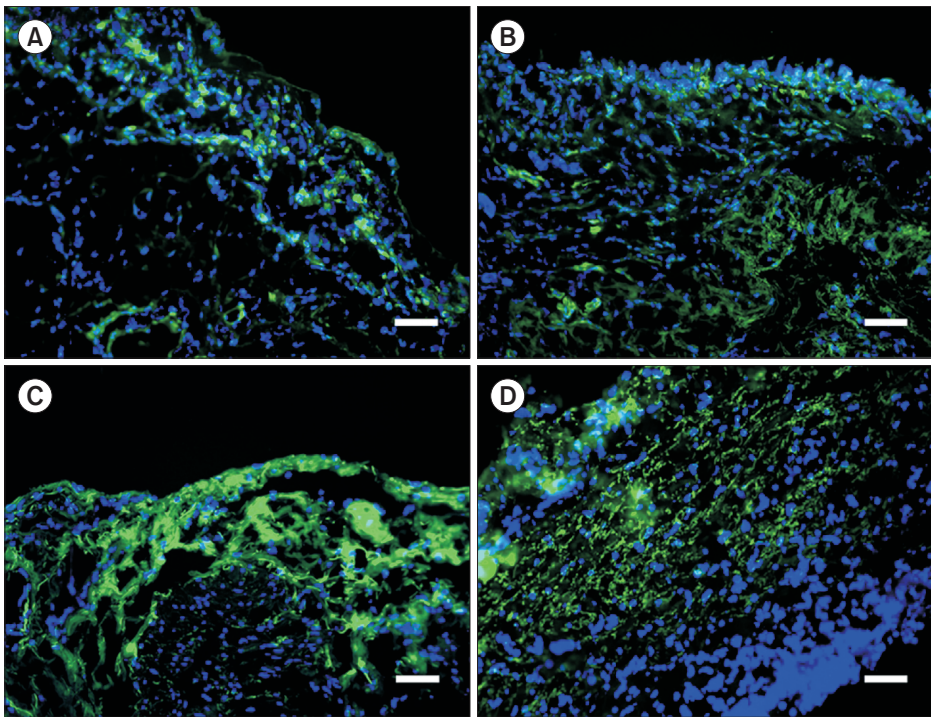
Fig. 1. Immunofluorescence staining of uroplakin-Ib and uroplakin-III. (A) Uroplakin-Ib expression in urothelium in control. (B) Uroplakin-Ib expression in urothelium in interstitial cystitis/bladder pain syndrome (IC/BPS). (C) Uroplakin-III in urothelium in control. (D) Uroplakin-III in urothelium in IC/BPS. Scale bars: 100 μm.
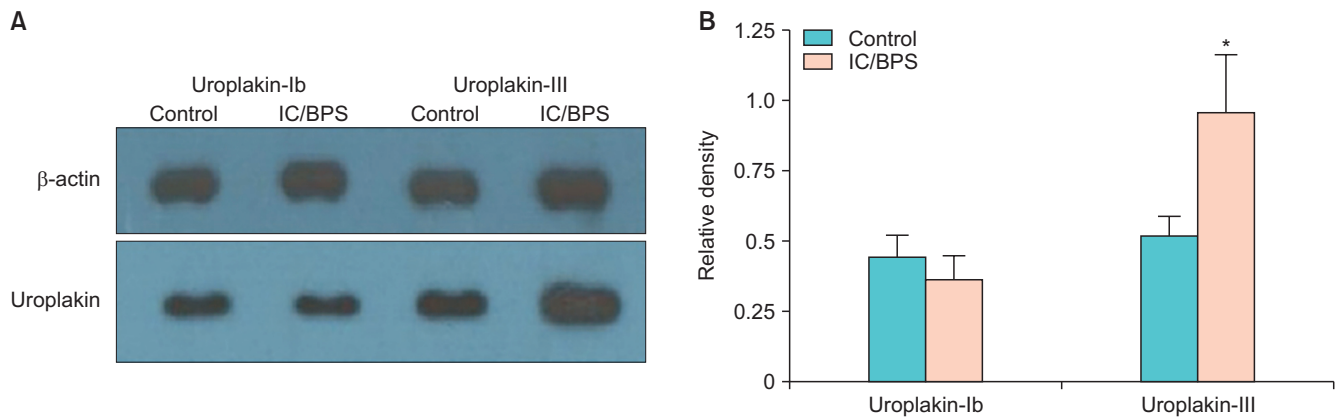

Fig. 2. Uroplakin expression levels. (A) Western blot analysis of uroplakin-Ib and uroplakin-III in urothelium in control and interstitial cystitis/bladder pain syndrome (IC/BPS). (B) Densitometric analysis relative to beta-actin. Data expressed as mean±standard deviation. Asterisk indicates significant difference between control and IC/BPS.

activity and decreased bladder pressure values required to initiate voiding compared with wild-type mice [13]. On the basis of these findings, previous studies identified a relationship between UP-III and IC/BPS. Lv et al. [14] reported that UP-III was significantly and quantitatively decreased in the bladder of IC/BPS patients compared with the control group, although their study made no distinction between the ulcerative and nonulcerative types of IC/BPS. Decreased UP-III levels and increased urothelial permeability in the bladder of a chemically induced IC animal model could be corrected by the intravesical administration of medication to inhibit inflammation and protect the mucosa [15]. These results also support evidence showing that a lack of UP-III changes uro-

thelial permeability and could induce IC/BPS. However, our study showed that UP-III was increased in nonulcerative lesions in patients with ulcerative IC/BPS. Although we did not measure the level of UP in the ulcerative lesion, we can assume that UP-III levels might be decreased at the ulcerative lesion where the urothelium is disrupted; indeed, previous studies have reported significantly decreased UP expression levels in ulcerative portions [16,17]. In our study, UP-III elevation in the non-ulcerative portion could be attributed to feedback regulation of UP-III reduction in the ulcerative portion. In contrast, Zeng et al. [6] showed that expression levels of UP-Ia, Ib, II, and III were significantly lower in urothelium obtained separately from ulcerative lesions in

ulcerative IC after 5 minutes of hydrodistension. This difference may have resulted from the different conditions of the enrolled patients. We obtained urothelial samples from patients who underwent augmentation ileocystoplasty a certain period of time after receiving other treatments for IC/BPS (including hydrodistension), but the samples in Zeng et al. [6]'s study were all obtained shortly after hydrodistension. Although the differences in urothelial changes in IC according to treatment duration are not yet clear, the results of a previous study showing changes in UP-III with stretch duration [18] suggest that differences may exist in the structure or neurotransmitter expression of the urothelium depending on the duration of bladder stimulation.

Although another previous study showed that the expression of UP-Ib differs in the bladders of ulcerative IC/BPS patients [19], our study did not find a significant difference in UP-Ib expression in ulcerative IC/BPS compared with that in the control group patients. UP-Ib is characterized by being able to get out of the endoplasmic reticulum and move to the plasma membrane alone. On the other hand, UP-III can reach the plasma membrane only when it is combined with UP-Ib [20]. It is unclear why UP-Ib does not differ in ulcerative IC/BPS, whereas UP-III does; it seems that the different cellular characteristics of UP-Ib and UP-III may be involved.

This study had some limitations. We did not conduct urodynamics and therefore could not reflect on any changes in UP according to bladder functional parameters in IC/BPS. In addition, because the patients enrolled in this study had been treated by several different methods before undergoing augmentation ileocystoplasty, the patients may have had true biological differences in their bladders compared with IC/BPS patients who had never received any previous treatment. However, in the current situation in which the diagnostic criteria of IC/BPS are still uncertain, the inclusion of patients without IC/BPS could be minimized by selecting patients with severe and chronic symptoms that require ileocystoplasty and also those with clearly ulcerative lesions on cystoscopy. One other limitation is that we analyzed only UP-Ib and UP-III in the urothelium. Analysis of other biomarkers in tissue or urine is necessary to support our findings and for application of our findings to the diagnosis of IC/BPS. Besides, we enrolled a relatively small number of patients, especially in the control group, and included a wide range of patients of both sexes without distinguishing gender differences in clinical characteristics. Further research considering these issues should be required in the future. We targeted IC/BPS patients with severe and chronic symptoms requiring ileocystoplasty; thus, anatomical and functional

differences between men and women in the results of this study would not be significant.

CONCLUSIONS

Expression levels of UP in the urothelium may change in the presence of IC/BPS. In this study, UP-III was significantly upregulated in patients with ulcerative IC/BPS. Because the pathophysiology of IC/BPS involves more than just urothelial factors, this result is insufficient to be used as a definite diagnosis of IC/BPS. However, UP-III is a potential biomarker for the diagnosis of ulcerative IC/BPS.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

AUTHORS' CONTRIBUTIONS

Research conception and design: Joon Chul Kim. Data acquisition: Kyu-Sung Lee and Joon Chul Kim. Data analysis and interpretation: Kang Jun Cho and Kyu-Sung Lee. Statistical analysis: Jin Bong Choi and Jun Sung Koh. Drafting of the manuscript: Kang Jun Cho. Critical revision of the manuscript: Kang Jun Cho. Supervision: Joon Chul Kim. Approval of the final manuscript: Joon Chul Kim.

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