Stress Related Induction of Bladder Urothelial Changes in Mice: A New Murine Model

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Abstract

Introduction and objective: Painful Bladder Syndrome (PBS) is a chronic, painful inflammation of the bladder wall. The cause of PBS is still unknown but physiological stress may play a role, and although there are many treatments available to relieve the symptoms there is no cure at this time. The lack of curative treatment modalities is further hampered by the lack of representative in vivo models. In an effort to establish an in vivo model of PBS, the effect of chronic mild stressors on normal mouse bladders was evaluated for the effect on both mast cells and urothelium thickness.

Methods: Forty male mice were exposed a series of random stressors daily (Cage Tilt, Damp Sawdust, No Sawdust, Social Stress, and Varying Light/Dark Cycles). After 16 weeks the mice were sacrificed and the bladders were prepared for pathologic investigation. The urinary bladders were formalin-fixed, paraffin-embedded, and evaluated using routine light microscopy with hematoxylin and eosin, giemsa, and PAS stained sections. Urothelial and detrusor muscle mast cell numbers were evaluated by averaging ten representative 200x fields in the Giemsa section. Urothelial thickness was evaluated by averaging six representative regions in the PAS section. The control and test groups were statistically compared using the non-parametric Mann-Whitney method.

Results: Urothelium was found to be significantly decreased in thickness when comparing the test to control mice (p = 0.0041). Additionally, the quantity of urothelial/sub mucosal mast cells was increased in the test versus control mice (p< 0.0001). No significant difference was observed in the quantity of detrusor mast cells.

Conclusion: The absence of effective and representative models of PBS has severely hindered the pursuit of curative treatments modalities for this disease. The significant reduction in both urothelial mast cells and in the thickness of the urothelium suggest that chronic multiple stressors may be effective in inducing bladder urothelial changes in mice. This data may provide investigators with an effective in vivo mouse model of PBS.

INTRODUCTION

Interstitial cystitis/painful bladder syndrome (IC/PBS) is a chronic inflammatory disorder characterized by bladder pain and lower urinary tract symptoms. Patients with IC/PBS report a significantly worse quality of life, including symptoms of depression/anxiety and sexual dysfunction [1]. Chronic stressors have been identified as a common characteristic in many patients diagnosed with IC/PBS, with multiple studies showing a significant relationship between chronic stress and worsening or exacerbation of IC/PBS symptoms [2,3]. Unfortunately, IC/PBS remains a challenging condition to both diagnose and treat. Much of this difficulty stems from poor understanding of the etiology and pathogenesis of the disease. This is in part due to a lack of good in vivo animal models to assist researchers in developing new diagnostic tests and therapies.

While IC/PBS is primarily a clinical diagnosis, previous research has identified physiologic and structural changes in the bladders of many of these patients that may help support the diagnosis. Research is ongoing, building on the multiple inflammatory, immune and pathological markers that have already been identified. A major focus of past research on IC/PBS was on the gross and/or histologic changes, primarily from the appearance of the bladder on cystoscopy and tissue samples from biopsies. Classic findings on cystoscopy include Hunner’s ulcers in the ulcerative type or diffuse glomerulations. While the clinical value of bladder biopsies remains controversial, it is still sometimes utilized to exclude other disease processes and still may be used to support the diagnosis [4].

There are various histologic changes described in some patients with IC/PBS. One well-described feature is an increased mast cell quantity [5,6]. In a recent review of the literature by Theoharis et al., they identified at least 26 human studies and 8 animal models showing primarily increased mast cell involvement [7]. While some studies have shown the mast cell density to be greater in the detrusor muscle [8], others have shown significant mucosal and sub mucosal involvement and/or de granulation [9]. In addition to mast cell involvement, multiple urothelial changes have been identified in the bladders of patients with IC/PBS including chronic inflammation [10] and a dysfunctional epithelium or GAG layer [11]. Additionally, denuding or thinning of the epithelium is an important histologic finding in the bladders of some patients with IC/PBS [10,12] and has led to the classification of ulcerative and nonulcerative subtypes.

of interstitial cystitis. This denuding is more pronounced the ulcerative type, and characterizes the classic finding of Hunner's ulcers on cystoscopy [13].

In this study we examined a novel murine model for stress induced urothelial changes based on the known relationship of IC/PBS to chronic stress. Our hypothesis is that application of chronic stress will lead to known pathologic and histological changes consistent with IC/PBS, such as increased number of mast cells and urothelial thinning.

MATERIALS AND METHODS

Forty male mice were initially obtained for the study. Balb/c male mice (6-7 weeks old) were received and acclimatized for two weeks. The animals were fed rodent chow and had ad libitum exposure to drinking water. Thirty (30) mice were randomized to the test (stress) group and 10 mice randomized to the control group. However, one test mouse died prior to starting the experiment, leaving us with 29 test mice.

The test mice were then exposed to a series of random stressors daily for 16 weeks. The stressor protocol was based on the work of Ducotter and Belzung [14] and consisted of the following stressors:

1. Damp bedding – 10 oz. of water was added to each standard cage with bedding lasting from 1 to 3 hours.
2. Bath – Bedding was removed and 12 oz. of water was added to empty cage for the duration of 15 minutes to 3 hours.
3. Each cage was tilted to 45 degrees with and without bedding for a period of 1 to 3 hours.
4. Social stress – each mouse was placed in the cage of his neighbor, while the neighboring mouse is placed in its neighbor’s cage.
5. No bedding lasting from 1 hour to 3 hour
6. Succession of light/dark cycles, every 15 to 30 minutes.

Control group (n = 10) animals were housed in a separate, quiet room in the animal facility. The Chronic Stress group (CS, n = 29) underwent a series of unpredictable chronic mild stressors

After 16 weeks of daily chronic stress, the mice were sacrificed and the bladders removed for histological analysis.

The mouse bladders were formalin fixed and paraffin embedded prior to routine light microscopy. Several stain types were prepared, including hematoxylin and eosin (H&E), Giemsa, and periodic acid-Schiff (PAS). Mouse bladder slides that were PAS stained were utilized for measurement of the Urothelium thickness. Giemsa stained slides were used to quantify the number of urothelial/submucosa and detrusor mast cells. H&E staining was prepared as an alternative stain to measure Urothelium thickness, but was not utilized for the study.

Using 200x magnification on the Giemsa stained slides, urothelial mast cells were quantified by averaging ten random high power fields, not counting the mast cells in the detrusor muscle (Figure 1). The procedure was then repeated, this time counting the total mast cells in the detrusor muscle while not counting those within the epithelium/submucosa. We were unable to successfully stain tissue for mast cell quantity on one of the test samples, leaving n = 28 for mast cell quantity.

Urothelium thickness was measured by first calibrating the microscope at 100x using a calibrated micrometer. Using PAS slides, epithelium thickness was measured in six representative fields from each sample and a mean calculated on a spreadsheet.
All results were entered into a Microsoft Excel spreadsheet. The control and test groups were statistically compared using the non-parametric Mann-Whitney method [15].

RESULTS

The bladder epithelium was significantly decreased in thickness when comparing the stressed to control mice (p = 0.0041). Additionally, the quantity of urothelial and sub mucosa mast cells was elevated in the test versus control mice (p<0.0001). There was no significant difference observed in the quantity of detrusor mast cells between test and control mice. These results are summarized in the Table 1.

DISCUSSION

The significant reduction in both epithelial mast cells and in the thickness of the bladder epithelium demonstrated in our murine model suggest that the application of chronic stressors may be effective for inducing IC/PBS changes in mice. This would be consistent with other recently published data that also suggest chronic stress may be an effective animal model for inducing IC/PBS. For example, Lee et al., found chronic psychological stress induced sustained bladder hyper algesia in anxiety-prone rats [16]. In another recent study, male rats were exposed to stressors over 7 days resulting in increased expression of TRPV4, an ion channel that had previously been implicated as pathologic in patients with IC/PBS [17]. Merrill et al., also applied stress to male rats then evaluated bladder function using intravesical infusion of saline. They observed significantly increased voiding frequency in their model [17].

While most IC/PBS animal models to date evaluate for physiologic changes as measured by gene expression, molecular markers or protein detection, there are several animal models evaluating histologic changes consistent with IC/PBS. For example, Golubeva et al., published a cyclophosphamide induced mouse model for IC/PBS, observing for changes in mast cell quantity and urothelial thickness in addition to expression of urothelial receptors. In contrast to our finding of urothelial thinning, the epithelium was found to be hyper plastic in their model [18]. However, a recent mouse model developed by Keay et al., did show findings consistent with our study with epithelial thinning and inhibition of repair by antiproliferative factor (AFP) following instillation of acetic acid [19]. All of these studies and our own emphasize the importance of further research into a stress induced model of bladder urothelial changes which may be similar to what is seen with IC/PBS.

This study does have some important limitations. First, the distribution of test and control samples should ideally be more equally distributed as sample size may significantly increase standard deviations. Second, observer bias could potentially have been introduced, as there was no blinding during the microscopic analysis of the slides and the measured variables relied on the analyst’s determination of “representative” high power fields. However, this could easily be addressed in future studies by blinding the analyst. Finally, less bias might be introduced if imaging software was utilized in urothelial measurements and/or mast cell quantification.

Despite the limitations, our mouse model also has some advantages over other animal models that have been described to date. Mouse models are more readily available than feline or other animal models. Additionally, the other described rodent models above utilized shorter durations of stress [16,17]. Finally, our model doesn’t rely on difficult or expensive procedures such as daily instillations of intravesical agents [16,17] complex laboratory tests, or cell cultures. Additionally, our model provides the basic framework for numerous future studies. For example, additional variables such as measurements of inflammatory cells, neural or vascular proliferation, autoimmune pathways or previously identified gene expression could be evaluated in mice following the application of chronic stress. If validated, this model could potentially be used to test current and future therapies for IC/PBS including bladder instillations, medications and lifestyle changes.

CONCLUSION

Stress and IC/PBS have a known relationship. The lack of animal models incorporating stress for IC/PBS has attenuated the development of curative treatments for this disease. The significant reduction in both urothelial/sub mucosa mast cells and in the thickness of the epithelium demonstrated in our murine model suggest that chronic multiple stressors may potentially be effective in inducing urothelial changes in mice. This data may provide investigators with an effective in vivo mouse model of IC/PBS from which to base further research.

REFERENCES

5. Sant GR, Kempuraj D, Marchand JE, Theoharis TC. The mast cell in interstitial cystitis: role in pathophysiology and pathogenesis.

| Table 1: Quantity of urothelial and sub mucosa mast cells in mouse bladder test samples. |
|---------------------------------|---------|---------|---------|---------|
| Urothelial Mast Cells (per 200x field; p = 0.0041) | Mean ± Std Dev | Median | Min | Max |
| Control (n = 10) | 0.03 ± 0.09 | 0.00 | 0.00 | 0.30 |
| Test Group (n = 28) | 0.31 ± 0.43 | 0.20 | 0.00 | 2.10 |
| Detrusor Muscle Mast Cells (per 200x field; p = 0.5731) | Mean ± Std Dev | Median | Min | Max |
| Control (n = 10) | 0.45 ± 0.64 | 0.16 | 0.00 | 1.90 |
| Test Group (n = 28) | 0.53 ± 0.58 | 0.30 | 0.00 | 2.00 |
| Urothelium Thickness (p<0.0001) | Mean ± Std Dev | Median | Min | Max |
| Control (n = 10) | 583 ± 114 µ | 570 µ | 473 µ | 840 µ |
| Test Group (n = 29) | 389 ± 66 µ | 393 µ | 267 µ | 513 µ |


