Mechanisms of Visceral Organ Crosstalk: Importance of Alterations in Permeability in Rodent Models

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Abbreviations and Acronyms

DAI = disease activity index
FITC-4 = fluorescein isothiocyanate-dextran
IBS = irritable bowel syndrome
IC = interstitial cystitis
Isc = short circuit current
OVX = ovariectomized
PBS = painful bladder syndrome
PD = potential difference
TEER = transepithelial electrical resistance
TNBS = trinitrobenzene sulfonic acid

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Purpose: The pathophysiology of painful bladder syndrome is poorly understood. However, there is evidence of female predominance and comorbidity with irritable bowel syndrome. Our hypothesis is that cross-sensitization between bladder and colon is due to altered permeability in 1 organ, which affects the other organ.

Materials and Methods: Experiments were performed in anesthetized, ovariectomized female rats. In separate groups protamine sulfate was infused in the bladder or trinitrobenzene sulfonic acid was infused in the colon. Untreated rats served as controls. Bladder and colonic tissue were harvested from all rats 1, 3 and 5 days after treatment. Permeability was assessed in vitro in Ussing chambers by measuring transepithelial electrical resistance and macromolecular flux of fluorescein isothiocyanate-dextran.

Results: Exposing the bladder to protamine sulfate induced a significant decrease in bladder transepithelial electrical resistance and an increase in the translocation of fluorescein isothiocyanate across the tissue compared to controls at 1 and 3 days ($p < 0.05$). Colonic tissue from rats with enhanced bladder permeability showed a significant decrease in transepithelial electrical resistance and increase in fluorescein isothiocyanate compared to untreated controls at all time points ($p < 0.05$). Conversely when colonic permeability was increased with trinitrobenzene sulfonic acid, we observed an increase in bladder permeability in the absence of any changes to the bladder urothelium.

Conclusions: Changes in epithelial permeability may represent a novel mechanism for visceral organ crosstalk. It may explain the overlapping symptomology of painful bladder syndrome and irritable bowel syndrome.

Key Words: urinary bladder; colon; cystitis, interstitial; irritable bowel syndrome; inflammation

Painful bladder syndrome or IC is a chronic pain condition that affects almost 1 million people in the United States, of whom the majority are women. PBS/IC is characterized by frequent urination, increased urgency and pain associated with bladder filling.[1] PBS/IC patients often experience a variety of other pelvic pain disorders, including functional bowel disorders such as IBS. As many as 30% to 50% of patients diagnosed with IBS show symptoms of PBS while as many as 40% diagnosed with PBS have symptoms that fulfill the criteria for IBS.[2,3]

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In support of cross-communication between visceral organs experiments have demonstrated that colonic irritation is capable of producing irregular micturition patterns such as early onset of micturition and enhanced urethral sphincter activity in a rat model.[4] In addition, there is evidence that active colonic inflammation induces abnormalities in bladder detrusor muscle contractility[5] and can result in vascular permeability in the bladder taken from female rats.[6] Conversely bladder irritation results in increased visceral sensitivity to colonic stimulation.[7]

The mechanisms of the overlap of symptomatology in patients with PBS and IBS are poorly understood. However, visceral organ communication may be a contributing variable. Evidence suggests that visceral organ crosstalk may be due to the convergence of sensory neural pathways in the dorsal root ganglion, spinal cord and/or brain.[8] Specifically dual labeling studies have revealed that approximately 3% to 15% of afferent nerve fibers innervating the bladder and colon are common to both structures.[9] In the lumbosacral region of the spinal cord approximately 30% of neurons respond to bladder and colorectal stimulation.[10] Current evidence has also pointed to sensitization of afferent neurons at the dorsal root ganglion as well as lumbosacral neurons that become hyperexcitable following colonic inflammation.[11] Thus, it is possible that afferent sensitization may have a pivotal role in visceral organ cross-communication.

The possibility exists that activation of afferent nerves in response to mucosal damage may be the result of increased epithelial permeability, allowing foreign substances to have direct access to the visceral sensory neurons. In support of this idea previous studies have shown that inflammation of the bladder can disrupt the integrity of the bladder within 24 hours and cause a marked reduction in TEER.[12,13] Similarly previous studies have shown that damage to the colon via TNBS increases colonic permeability and afferent neuron activity in the colon.[14–17]

Given that PBS and IBS are characterized by the presence of minimal to low grade inflammation, we performed this study to investigate the effect of acute disruption of the bladder urothelium or colonic mucosa on bladder and colonic permeability. Our aim was to test the hypothesis that enhanced epithelial permeability represents a novel mechanism for visceral organ crosstalk and may explain the overlapping symptomatology of PBS and IBS.

MATERIALS AND METHODS

Animal Model

Female OVX Sprague Dawley® rats weighing 220 to 250 gm were obtained from Charles River Laboratories, Wilmington, Massachusetts. All animals had free access to food and water, and were acclimated to facility housing for a minimum of 1 week before experimentation. The protocol was approved by the University of Oklahoma Health Sciences Center institutional animal care and use committee (animal protocol No. 11-162).

Study 1: Protamine Sulfate Administration in Bladder

Rats were anesthetized with isoflurane (3%) and oxygen for approximately 10 minutes and protamine sulfate was instilled transurethrally as previously described.[13] The bladder and colon were harvested 24 hours, and 3 and 5 days after the infusion of protamine sulfate. Non-catheterized OVX female rats served as naïve controls.

Bladder Damage Assessment

Segments of bladder were isolated, fixed and blocked. Sections (10 μm) were histologically evaluated by a board certified animal pathologist blinded to treatment for signs of inflammation and damage.

Study 2: TNBS Administration in Colon

Rats were fasted overnight with free access to water, removed from the animal facility and brought to the laboratory. They were briefly anesthetized with isoflurane (3%) with a steady supply of oxygen. While sedated, the rats received an enema containing a solution of 50 mg/ml TNBS diluted in 25% ethanol and 25% saline. The volume of TNBS solution infused in the colon was adjusted so that each rat received 50 mg/kg TNBS. Controls were infused with 100% saline.

Disease Activity Index

To determine the severity of TNBS induced colonic inflammation the rats were weighed and stools were graded for 5 consecutive days after TNBS infusion. DAI was calculated as previously described.[18] The presence of blood in the feces was tested using an occult blood indicator test (Beckman Coulter, Fullerton, California).

Colonic Damage Assessment

Segments of colon tissue (approximately 1 × 1 cm) were harvested from each rat. Tissues were embedded in 10% buffered formalin, processed, embedded in paraffin and sectioned at 5 μm. They were stained with hematoxylin and eosin, and assessed under a light microscope for inflammation by a board certified animal pathologist blinded to treatment. Inflammatory parameters were assessed using a previously validated method.[18]

Bladder and Colonic Permeability Measurements

Following tissue isolation the colon and bladder were opened longitudinally and mounted in biopsy perfusion chambers (bladder) or modified Ussing chambers (colon). Organs were bathed in oxygenated Krebs solution at 37C. Permeability was assessed electrophysiologically by measuring TEER. PD and Isc were recorded and used to calculate TEER. Ohm's law was applied to PD and Isc ($I = PD/R$), where R represents resistance.

Permeability was also assessed via the movement of the macromolecular marker FITC-4. In these experiments FITC-4 was added to the urothelial (bladder) or mucosal (colon) side of preparations to determine macromolecular flux using fluorescence spectroscopy.