Intravesical Botulinum Toxin A Administration Inhibits COX-2 and EP4 Expression and Suppresses Bladder Hyperactivity in Cyclophosphamide-Induced Cystitis in Rats

Yao-Chi Chuanga,*, Naoki Yoshimurab, Chao-Cheng Huangc, Moya Wu a, Po-Hui Chianga, Michael B. Chancellord

a Department of Urology, Chang Gung Memorial Hospital, Kaohsiung Medical Center, Chang Gung University College of Medicine, Kaohsiung, Taiwan
b Department of Urology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, United States
c Department of Pathology, Chang Gung Memorial Hospital, Kaohsiung Medical Center, Chang Gung University College of Medicine, Kaohsiung, Taiwan
d Department of Urology, William Beaumont Hospital, Royal Oak, Michigan, United States

Article info

Article history:
Accepted May 6, 2008
Published online ahead of print on May 20, 2008

Keywords:
Botulinum toxin
COX2
EP4
Bladder
Interstitial cystitis

Abstract

Background: Cyclooxygenase 2 (COX-2) elevation and subsequent prostaglandin E2 (PGE2) production play a major role in bladder inflammation and hyperactivity. EP4 receptor, a subtype of PGE2 receptors, mediates tissue inflammation and hypersensitivity.

Objective: To investigate the effect of intravesical botulinum toxin A (BoNT-A) on COX-2 and EP4 expression in cyclophosphamide (CYP)-induced cystitis in rats.

Design, setting, and participants: Experimental (N = 40) and control animals (N = 20) were injected with CYP (75mg/kg intraperitoneally) or saline on days 1, 4, and 7. BoNT-A (1 ml, 20 unit/ml) or saline were administered into the bladder and retained for 1 h on day 2.

Intervention: Waking cystometrograms (CMGs) were performed. Bladder and L6 and S1 spinal cord were harvested on day 8.

Measurements: CMG parameters, histology, and COX-2 and EP4 expression by immunostaining or western blotting were measured.

Results and limitations: CYP induced increased bladder inflammatory reaction, bladder hyperactivity, and COX-2 and EP4 expression in the bladder and spinal cord. The CYP effects were suppressed by BoNT-A treatment. BoNT-A treatment decreased inflammatory reaction (56.5% decrease), COX-2 expression (77.8%, 61.7%, and 54.8% decrease for bladder, L6, and S1 spinal cord, respectively), EP4 expression (56.8%, 26.9%, and 84.2% decrease for bladder, L6, and S1 spinal cord, respectively), and suppressed bladder hyperactivity (intercontraction interval, 107% increase and contraction amplitude, 43% decrease).

Conclusions: CYP injection activated COX2 and EP4 expression in the bladder and spinal cord and induced bladder inflammation and hyperactivity, which effects were suppressed by BoNT-A treatment. These findings suggest a potential benefit of EP4-targeted pharmacotherapy and BoNT-A treatment for bladder inflammatory conditions.

© 2008 European Association of Urology. Published by Elsevier B.V. All rights reserved.

* Corresponding author. 123 Ta Pei Road, Niao Song Hsiang, Kaohsiung Hsien, Taiwan.
Tel. +886 7 7317123; Fax: +886 7 7318762.
E-mail address: Chuang82@ms26.hinet.net (Y.-C. Chuang).
1. Introduction

Interstitial cystitis/painful bladder syndrome (IC/PBS) is a debilitating clinical syndrome characterized by bladder pain, frequency, and urgency in the absence of any identifiable pathologic factors [1,2]. Although the etiology and pathogenesis of IC/PBS are still undetermined, activation of bladder afferent nerves and chronic inflammation of the urinary bladder are common mechanisms of IC/PBS symptoms [1,3].

Cyclooxygenase (COX)-2 is a key enzyme in the conversion of arachidonic acid to proinflammatory prostaglandins, in particular, prostaglandin E₂ (PGE₂), which have been thought to play a major role in the process of inflammation and hypersensitivity [4,5]. Previous cystitis models in rats have revealed that increased COX-2 expression and subsequent PGE₂ production affect micturition reflex and induce bladder pain [5,6]. Recent experiments on Freund’s adjuvant-induced somatic pain demonstrated that activation of EP₄ receptor, a subtype of PGE₂ receptors, has contributed to inflammatory pain and hypersensitivity [4]. Therefore, it would be interesting to explore the link between COX-2 and EP₄ levels with cystitis.

Botulinum toxin A (BoNT-A) was originally known to block acetylcholine release at neuromuscular junctions and have therapeutic effects on muscular hypercontraction [6–8]. The effect of BoNT-A on sensory pathways is supported by data from preclinical models of bladder pain, in which intravesical application of BoNT-A significantly reduced pain responses and inhibited calcitonin gene-related peptide (CGRP) release from afferent nerve terminals [9]. Recent animal studies in rats by Vemulakonda et al [10] also suggest that BoNT-A may inhibit afferent neurotransmission and suppress bladder hyperactivity induced by CYP. Taken together, these results present an explanation for its clinical efficacy in the treatment of human chronic bladder inflammation and bladder pain [8,11].

In the present study, we explored the effects of BoNT-A on the expression of COX-2 and EP₄ in an experimental rat model of chronic cystitis induced by intraperitoneal cyclophosphamide (CYP) injection. We hypothesized that BoNT-A might suppress COX-2 and EP₄ expression in the bladder and spinal cord and reduce the CYP-induced bladder hyperactivity and inflammation.

2. Material and methods

2.1. Cyclophosphamide (CYP) injection

All experimental procedures were performed on female Sprague-Dawley rats (220–270 gm, N = 60) and reviewed and approved by the Institutional Animal Care and Use Committee before the study began. Chronic cystitis was induced by intraperitoneal injection of CYP, which is metabolized to acrolein, an irritant eliminated in the urine [5]. CYP (75 mg/kg; ip) or a corresponding volume of saline was injected on days 1, 4, and 7.

2.2. Botulinum toxin A (BoNT-A) administration

On day 2, PE-50 tubing (Clay-Adams, Parsippany, NJ) was inserted into the bladder through the urethra and tied in place by a ligature around the urethral orifice under halothane anesthesia. The bladder was emptied of urine, and filled with BoNT-A (1 ml, 20u/ml in saline, Allergan, Irvine, CA) or sterile saline for 1 h through the catheter.

2.3. Cystometrogram (CMG)

On day 8, the animals (N = 6, for each group) were anesthetized with halothane and a lower midline abdominal incision was performed. A PE-50 tube with the end flared by heat was inserted into the bladder dome and secured in place with a 6-0 nylon purse-string suture. The distal end of the tubing was tunneled subcutaneously, and externalized at the back of the neck. The wound was closed in layers. After recovery from anesthesia, the animals were gently restrained and the suprapubic catheter was connected via a three-way stopcock to a pressure transducer and to a syringe pump for recording intravesical pressure and for infusing saline (0.08 ml/min) into the bladder to elicit repetitive voiding. The amplitude and intercontraction interval (ICI) of reflex bladder contractions were recorded. Measurements in each animal represented the average of 3–5 bladder contractions.

2.4. Transcardiac perfusion

On day 8, some animals (N = 7, for each group) were deeply anesthetized and sacrificed via transcardiac perfusion, first with Krebs buffer followed by 4% paraformaldehyde fixative. The animals were then dissected to harvest the bladder.

2.5. Histology and immunohistochemistry

The bladder was fixed in 10% buffered formaldehyde for 24–48 h, and then embedded in paraffin. The bladder tissue for histology was cut in 3–μm thick pieces and stained with hematoxyline and eosin. The inflammatory reaction of CYP-induced cystitis was graded by a score of 0–3 as follows: 0, no evidence of inflammatory infiltration or interstitial edema; 1, mild (few inflammatory cell infiltrates and little or no interstitial edema); 2, moderate (infiltration of moderate amount of inflammatory cell infiltrates and moderate interstitial edema); 3, severe (diffuse presence of large amount of inflammatory cell infiltrates and severe interstitial edema) [5,12].

Alternatively, the tissue sections of bladder for immunohistochemistry were cut into 3–μm sections, dewaxed in xylene, and rehydrated to distilled water through decreasing concentrations of alcohols. Antigen retrieval was achieved by pressure-cooking tissue sections immersed in 10M citrate buffer at pH 6.0. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide solution. Tissue sections were then incubated with rabbit anti-COX-2 polyclonal antibody (Cayman chemical, MI, USA, 1:500 dilution) or rabbit anti-EP₄ receptor polyclonal antibody (Cayman chemical, MI, USA, 1:1000 dilution) at +4°C over night. Sections were then washed in phosphate buffered saline (PBS) at pH 7.0 and incubated in BioGenex Super Enhancer Reagent for 20 min. After further washing in PBS, the sections were incubated for 30 min in BioGenex Polymer Horseradish Peroxidase Complex (LabTech Diagnostics, Kent, UK) followed by PBS wash. Slides were then developed with 3,3-diaminobenzidine chromogen (BioGenex DAB substrate, LabTech Diagnostics, Kent, UK) and counterstained with Mayer’s haematoxylin. Slides were then dehydrated through increasing concentrations of alcohol to xylene and coveredslip-mounted with Entellan (Merck, Darmstadt, Germany).

2.6. Western blot analysis for COX-2 and EP4 expression

Some animals (N = 7, for each group) were deeply anesthetized and sacrificed without transcardiac perfusion. The bladder and L6 and S1
spinal cord were removed for Western blot analysis of COX-2 and EP4 expression according to the standard protocol (Amersham Biosciences) and our previous study [13]. The samples were homogenized in protein extraction solution (T-PER; Pierce Biotechnology) prior to sonication and purification. The amount of total protein was measured with the Bradford protein assay method (Bio-Rad Laboratories, Hercules, CA). SDS-polyacrylamide gel electrophoresis (PAGE) was performed using the buffer system of Laemmli. Briefly, an aliquot of the extracts equivalent to 30 μg protein was loaded onto 8% polyacrylamide gel, electrophoresed at a constant voltage of 100V for 1 h and transferred to Hybond-P PVDF Membrane (Amersham Biosciences). The membrane was blocked with blocking agent and then immunoblotted overnight at +4 °C with rabbit anti-COX2 polyclonal antibody (Cayman Chemical, MI, USA, 1:200 dilution) and mouse anti-β-actin monoclonal antibody (MBL, Nagoya, Japan, 1:2000 dilution) or rabbit anti-EP4 receptor polyclonal antibody (Cayman Chemical, MI, USA, 1:500 dilution). After wash, the membrane was incubated with secondary antibody using 5% defatted milk powder in TBS for 2 h at room temperature, using a horseradish peroxidase-linked antirabbit or antimouse immunoglobulin G. Western blots were visualized by enhanced chemiluminescence (ECL) detection system (Amersham Biosciences). The amount of β-actin was also detected as the internal control. Quantitative analysis was done using LabWorks Image Acquisition and Analysis software.

2.7. Statistical analysis

Quantitative data are expressed as means plus or minus standard error of mean. Statistical analyses were performed using one way ANOVA with Bonferroni posttests or Kruskal-Wallis with Dunn’s posttest where applicable, with \( p < 0.05 \) considered significant.

3. Results

3.1. CMG response to CYP and BoNT-A treatment

As shown in Table 1 and Fig. 1 (a,b), on day 8, CYP treatment induced increase of contraction amplitude (54.9%) and decrease of ICI (47.2%) compared to the control (saline injection) group. These CMG parameters reflect the bladder hyperactivity induced by CYP injection. However, the CYP effects were significantly suppressed by BoNT-A treatment (Table 1; contraction amplitude, 43.0% decrease, ICI, 107.0% increase). These results indicate the therapeutic effect of BoNT-A on bladder hyperactivity.

Table 1 – On day 8, effects of control, CYP, and CYP+BoNT-A on CMG parameters, including intercontraction interval and amplitude

<table>
<thead>
<tr>
<th></th>
<th>ICI (min)</th>
<th>Amplitude (cm H²O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Control</td>
<td>10.8 ± 1.2</td>
<td>21.3 ± 1.6</td>
</tr>
<tr>
<td>B: CYP+saline</td>
<td>5.7 ± 0.5</td>
<td>33.0 ± 3.1</td>
</tr>
<tr>
<td>C: CYP+BoNT-A</td>
<td>11.8 ± 1.7</td>
<td>18.8 ± 3.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>p value</th>
<th>ICI</th>
<th>Amplitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>A vs B</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>A vs C</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>B vs C</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

ICI = intercontraction interval; CYP = cyclophosphamide.

Data presented as means ± SE.

N = 6, for each group.

Bonferroni’s multiple comparison test.

3.2. Histologic response to CYP and BoNT-A treatment

As shown in Fig. 2, the CYP-treated group revealed mucosal sloughing, and edematous change in the bladder mucosa and submucosal layers associated with larger amount of inflammatory cells accumulation, as determined by the histopathologic evaluation of tissue section stained with hematoxylin and eosin, compared to the control group. These results suggest that CYP treatment has induced inflammatory changes. However, the CYP effects were significantly decreased by BoNT-A treatment (Table 2, total inflammatory score 56.5% reduction). These results indicate the therapeutic effect of BoNT-A on bladder inflammation.
3.3. COX-2 and EP4 expression on CYP and BoNT-A treatment

As shown in Fig. 3, CYP treatment induced an increase in COX2 (Fig. 3B) and EP4 (Fig. 3E) expression in the bladder mucosa region. Western blotting (Table 2 and Fig. 4) demonstrated that COX-2 protein level generally paralleled that of EP4 after CYP injection. COX-2 expression was 15.8-fold, 6.0-fold, and 3.1-fold increased at bladder, L6 spinal cord, and S1 spinal cord, respectively. EP4 expression was 3.7-fold, 6.7-fold, and 5.7-fold increased at bladder, L5 spinal cord, and L6 spinal cord, respectively. However, the CYP effects were significantly decreased by BoNT-A treatment (Fig. 4). COX-2 expression was 77.8%, 61.7%, and 54.8% reduction for bladder, L6 spinal cord, and S1 spinal cord, respectively. Reduction in EP4 expression was 56.8%, 26.9%, and 84.2% for bladder, L6 spinal cord, and S1 spinal cord, respectively.

4. Discussion

The present study demonstrated that the CYP injection in rats induced central sensitization with increased COX-2 and EP4 expression on CYP and BoNT-A treatment. As shown in Fig. 3, CYP treatment induced an increase in COX2 (Fig. 3B) and EP4 (Fig. 3E) expression in the bladder mucosa region. Western blotting (Table 2 and Fig. 4) demonstrated that COX-2 protein level generally paralleled that of EP4 after CYP injection. COX-2 expression was 15.8-fold, 6.0-fold, and 3.1-fold increased at bladder, L6 spinal cord, and S1 spinal cord, respectively. EP4 expression was 3.7-fold, 6.7-fold, and 5.7-fold increased at bladder, L5 spinal cord, and L6 spinal cord, respectively. However, the CYP effects were significantly decreased by BoNT-A treatment (Fig. 4). COX-2 expression was 77.8%, 61.7%, and 54.8% reduction for bladder, L6 spinal cord, and S1 spinal cord, respectively. Reduction in EP4 expression was 56.8%, 26.9%, and 84.2% for bladder, L6 spinal cord, and S1 spinal cord, respectively.

Table 2 – On day 8, effects of control, CYP, and CYP+BoNT-A on inflammation (inflammatory cell scoring and edema scoring), and COX-2 and EP4 expression (band density)

<table>
<thead>
<tr>
<th></th>
<th>Edema</th>
<th>Inflammatory cell</th>
<th>Bladder COX-2</th>
<th>Bladder EP4</th>
<th>L6 COX-2</th>
<th>S1 COX-2</th>
<th>L6 EP4</th>
<th>S1 EP4</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Control</td>
<td>0.4 ± 0.2</td>
<td>0.3 ± 0.2</td>
<td>1.0 ± 0.0</td>
<td>1.0 ± 0.0</td>
<td>1.0 ± 0.0</td>
<td>1.0 ± 0.0</td>
<td>1.0 ± 0.0</td>
<td>1.0 ± 0.0</td>
</tr>
<tr>
<td>B: CYP+saline</td>
<td>2.6 ± 0.2</td>
<td>2.0 ± 0.3</td>
<td>15.8 ± 5.6</td>
<td>3.7 ± 0.8</td>
<td>6.0 ± 2.0</td>
<td>3.1 ± 0.6</td>
<td>6.7 ± 1.8</td>
<td>5.7 ± 2.2</td>
</tr>
<tr>
<td>C: CYP+BoNT-A</td>
<td>1.1 ± 0.3</td>
<td>0.9 ± 0.1</td>
<td>3.5 ± 1.4</td>
<td>1.6 ± 0.4</td>
<td>2.3 ± 0.7</td>
<td>1.4 ± 0.4</td>
<td>4.9 ± 1.6</td>
<td>0.9 ± 0.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A vs B</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>A vs C</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>B vs C</td>
<td>&lt;0.05</td>
<td>&gt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

CYP = cyclophosphamide.
Data presented as means ± SE.
N = 7, for each group.
Dunn's multiple comparison test for edema and inflammatory cell scoring.
Bonferroni's multiple comparison test for COX-2 and EP4 expression.
EP4 expression in the spinal cord, in addition to peripheral sensitization of bladder inflammation and hyperactivity. These results indicated that there is interplay between bladder and spinal cord COX-2 and EP4 expression in chronic cystitis. Furthermore, elevation of COX-2 level generally paralleled that of EP4, which results suggest activation of EP4 contributes to the current cystitis model. Treatment with BoNT-A inhibited COX-2 and EP4 expression at the level of bladder and L6 and S1 spinal cord and suppressed bladder inflammation and hyperactivity. We concluded that BoNT-A might have effects on reducing peripheral inflammation and subsequent central sensitization through the modulation of COX-2 and the EP4 pathway.

CYP-induced cystitis has long been used as an acute cystitis model as well as a chronic cystitis model. The urinary bladder is the organ most affected by the toxic metabolite of CYP-acrolein, which is eliminated in the urine, stimulates capsaicin-sensitive bladder afferents, and induces neurogenic inflammation [5,10]. In agreement
with previous studies, the present experiments demonstrated that CYP treatment in rats induced bladder inflammation and hyperactivity, and increased COX-2 expression mainly in the bladder urothelial region [14]. Furthermore, the present studies expand these results by demonstrating up-regulation of EP4 expression in the bladder urothelium and L6 and S1 spinal cord, in accordance with up-regulation of COX-2 expression.

COX-2 is an inducible enzyme at the site of inflammation. The increased expression of COX-2 in the inflammatory bladder could lead to increased production of prostaglandins, particularly prostaglandin E2, which stimulate capsaicin-sensitive bladder afferents and produce localized pain and bladder hyperactivity [14–16]. Lecce et al demonstrated that inhibition of COX-2 prevents or reverses the urodynamic changes associated with bladder inflammation induced either by surgery, lipopolysaccharide, or CYP treatment [15]. There are four subtypes of PGE2 receptors (EP1 to EP4) that mediate the diverse effects of the prostanoid, based on their differences in signal transduction, tissue localization, and regulation of expression [4]. A previous study demonstrated that PGE2, via EP1 receptor, contributes to detrusor overactivity after bladder outlet obstruction [17]. The present cystitis model found that elevation of COX-2 expression generally paralleled that of EP4 in the bladder and spinal cord. The increased expression of COX-2 at inflammatory bladder could lead to increased production of PGE2, which might sensitize peripheral EP4 receptors and produce bladder hyperactivity. Furthermore, sensitization of sensory fibers in the bladder might activate central afferent pathways, and this assumption is supported by the present observation of concurrent increased expression of COX2 and EP4 in the L6 and S1 spinal cord. A previous study in a capsaicin-induced prostatitis model in rats also suggested that spinal processing of viscerosensory information is greatly facilitated [18]. COX2 and EP4 elevation in the spinal cord may play an important role in the development of hypersensitivity following peripheral stimulation.

Bladder urothelium and sensory axons in the bladder play an important role in the sensory transduction mechanisms modulating micturition, particularly in conditions of increased sensory nerve transmission following chronic inflammation or detrusor overactivity [19]. Various sensory mediators, including CGRP, substance P, ATP, and nerve growth factor (NGF), are released in response to noxious stimuli. BoNT-A, acting by cleaving the cytosolic translocation protein synaptosomal associated membrane protein (SNAP-25) and inhibiting the process of exocytosis, has been demonstrated to reduce the release of these neurotransmitters [8,9,19,20], and has therapeutic effects on detrusor overactivity as well as chronic inflammatory disorders, such as IC/PBS and chronic prostatitis [11,21–25]. COX-2 pathways contributed to increased spinal excitability during persistent peripheral inflammation. Our previous study demonstrated that BoNT-A affects on the inhibition of COX-2 expression from a peripheral organ, ie, the prostate, to the spinal cord, in an acute neurogenic prostatitis models [13,18]. The current study further extended the results by demonstrating that BoNT-A inhibited COX-2 expression in a chronic inflammatory model as well. By acting at the afferent C-fibers in the bladder, BoNT-A could block afferent inflow directed to the spinal cord and decrease central transport of substance P and neurotrophic factors [19]. Therefore, BoNT-A could block peripheral tissue inflammation and inflammatory cell accumulation and reduce COX-2 and EP4 expression in the peripheral organ and spinal cord.

Previous studies demonstrated that increased excitability of bladder C afferent neurons after CYP-induced chronic cystitis [26]. Klinger et al reported that COX-2 expression is significantly increased in the urothelium, and in nerve fibers in the suburothelial plexus [27]. The stimulating effects of CYP are mediated by the effects of inflammatory mediators such as ATP, NGF, and PGE2 on afferent terminals in the bladder wall. We suggest that BoNT-A effect on bladder afferent neurotransmission and urethral secretion and results in reduction of COX-2 and EP4 expression.

The molecular weight of BoNT-A was 150 kD, which was hard to get through the intact urothelium. Previous experiments have suggested that it is necessary to expose the bladder to protamine sulfate to increase epithelial permeability and increase the penetration of BoNT-A [8,9]. A recent report demonstrated that one injection of CYP induced acute and intermediate inflammation of the bladder [14], which conditions will increase permeability of urothelium. Therefore, the present study administered BoNT-A one day after CYP injection, without pretreatment with protamine sulfate, and still showed the pharmacologic effects of BoNT-A on the suppression of CYP-induced bladder inflammation and hyperactivity. Previous studies have shown the efficacy of BoNT-A on day 7, but not on day 3, in an acute cystitis model [9]. The effects of BoNT-A could be variable according to the different time point.

Vemulakonda et al demonstrated in a CYP-induced cystitis rat model that nonvoiding ICI increased by more than 10-fold in BoNT-A/CYP-treated animals compared with CYP-treated animals [10]. However, they did not find any modification in the frequency and amplitude of voiding contractions with BoNT-A treatment, whereas in the present study, such modifications are reported. Another study revealed that BoNT-A effectively decreases the effects of ATP-induced detrusor overactivity, both in the frequency and the amplitude of bladder contractions, but has no significant effects on capsaicin-induced overactive bladder [27]. The different doses of BoNT-A, duration of exposure to BoNT-A, and chemical inducers of cystitis might result in various degrees of effects of BoNT-A on sensory and motor function, and result in different responses on CMG parameters. Further study with the measurement of micturition pressure threshold, basal pressure, micturition volume, and nonvoiding ICI, or using organ bath experiments for evaluation of contraction and relaxation of the bladder by administering different pharmacologic agents and by creating dose-response curves, would be more informative.

Limitations of this study should be considered. We found that CYP induced volume reduction and inflammation of the
bladder and that the CYP effects were reduced in BoBT-A-treated rats. However, a more precise quantification of the observation is lacking. Further study with scoring of the COX-2 expression according to the intensity and extent of COX-2 staining, and comparison of the bladder weights among the three groups of treatment, would provide with a more robust demonstration [13,28]. In conclusion, intravesical BoNT-A administration blocked the CYP-induced bladder inflammation and hyperactivity and inhibited COX-2 and EP4 expression in the bladder as well as the spinal cord. These findings suggest a potential clinical benefit of EP4-targeted pharmacotherapy and BoNT-A treatment for bladder inflammatory conditions.

**Author contributions:** Yao-Chi Chuang had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study concept and design:** Chuang, Yoshimura.

**Acquisition of data:** Wu.

**Analysis and interpretation of data:** Wu, Chuang, Huang.

**Drafting of the manuscript:** Chuang.

**Critical revision of the manuscript for important intellectual content:** Chancellor, Yoshimura.

**Statistical analysis:** Wu, Chuang.

**Obtaining funding:** Chuang.

**Administrative, technical, or material support:** Chiang.

**Supervision:** Chancellor.

**Other (specify):** None.

**Financial disclosures:** I certify that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: Michael B Chancellor and Yao-Chi Chuang are consultants of Allergan.

**Funding/Support and role of the sponsor:** The National Science Council Taiwan provided the funding for this study.

**References**


Editorial Comment on: Intravesical Botulinum Toxin A Administration Inhibits COX-2 and EP4 Expression and Suppresses Bladder Hyperactivity in Cyclophosphamide-Induced Cystitis in Rats
Linda Vignozzi, Annamaria Morelli
Department of Clinical Physiopathology,
University of Florence, Florence, Italy
l.vignozzi@dfc.unifi.it

Interstitial cystitis (IC)—or painful bladder syndrome (BPS)—is a chronic bladder condition characterised by “the complaint of suprapubic pain related to bladder filling, accompanied by increased daytime and night-time frequency” [1]. IC has typical cystoscopic and histologic features; however, frequently the diagnosis is one of exclusion. IC is diagnosed after the physician has ruled out specific causes, such as urinary infection and malignancy. IC represents an important inflammatory disorder underlying overactive bladder (OAB), which is characterised by increased spontaneous myogenic activity, fused tetanic contractions, altered responsiveness to stimuli, and characteristic changes in smooth muscle ultrastructure.

Intravesical botulinum neurotoxin type A (BoNT/A), which has revolutionised the treatment of intractable symptoms associated with the neurogenic or idiopathic OAB, is being increasingly used in BPS/IC [2]. However, its mode of action in the human bladder remains largely unknown. BoNT/A has been found to inhibit the release of a number of neurotransmitters (including acetylcholine, adenosine triphosphate, and neuropeptides such as substance P) and to down-regulate the expression of purinergic and capsaicin receptors on afferent neurons within the bladder. In an experimental model of cyclophosphamide (CYP)-induced cystitis [3] it has been hypothesized that BoNT-A might suppress COX-2 and PGE2 receptor subtype (EP4) expression, reducing the induced bladder hyperactivity and inflammation. Another drug has recently been shown to ameliorate bladder dysfunction through a dual mechanism of action. A vitamin D receptor agonist, elocalcitol, which has well-known efficacy in reducing prostate growth and inflammation [4], inhibited the activation of the RhoA/Rho-kinase pathway (the “calcium sensitization pathway”) in the bladder muscular compartment, reducing spontaneous and inappropriate bladder contractility [5,6], and preventing voiding impairment and urinary retention.

In conclusion, preclinical studies like these, investigating the effect of current and emerging therapies, acting on the different pathophysiological factors underlying the bladder hyperactivity and inflammation, should be encouraged.

References


DOI: 10.1016/j.eururo.2008.05.008
DOI of original article: 10.1016/j.eururo.2008.05.007

---

Editorial Comment on: Intravesical Botulinum Toxin A Administration Inhibits COX-2 and EP4 Expression and Suppresses Bladder Hyperactivity in Cyclophosphamide-Induced Cystitis in Rats
Antonella Giannantoni
Department of Urology, University of Perugia, Policlinico Monteluce, Via Brunamonti 51, 06122 Perugia, Italy
agiantoni@libero.it

In this study, Chuang and coworkers investigated the effects of intravesical botulinum toxin A (BoNT-A) on the expression of cyclooxygenase (COX-2) and EP4, a subtype of prostaglandin E2 (PGE2) receptors, in cyclophosphamide (CYP)-induced cystitis in rats [1]. COX-2 is a key enzyme in the conversion of arachidonic acid to proinflammatory prostanooids, in particular PGE2, which play a major role in the process of inflammation and hypersensitivity. Recent observations with chemical cystitis models in rats have revealed that increased COX-2 expression and subsequent PGE2 production affect micturition reflex and induce bladder pain [2,3].

The authors of the study hypothesized that BoNT-A could suppress COX-2 and EP4 expression in the bladder and spinal cord and reduce the cyclophosphamide-
induced bladder hyperactivity and inflammation. Experimental and control animals were injected with CYP or saline. Awake cystometrograms were performed, and bladder and L6 and S1 spinal cord were further harvested. CMG parameters, histology, and COX-2 and EP4 expression were measured by immunostaining or western blotting.

The authors found that CYP induced increased bladder inflammatory reaction, bladder hyperactivity, and COX-2 and EP4 expression in the bladder and spinal cord. The CYP effects were suppressed by BoNT-A treatment, which decreased inflammatory reaction and COX-2 expression for the bladder, and L6 and S1 spinal cord. Furthermore, BoNT-A treatment also decreased EP4 expression into the bladder and L6 and S1 spinal cord, and suppressed bladder overactivity.

BoNT-A, acting by cleaving the cytosolic translocation protein synaptosomal associated membrane protein (SNAP-25) and inhibiting the process of exocytosis, has been demonstrated to reduce the release of several neurotransmitters, as SP, ATP, CGRP, NGF, inducing therapeutic effects on chronic inflammatory disorders, such as interstitial cystitis/painful bladder syndrome, and chronic prostatitis [4]. By demonstrating that BoNT-A inhibits COX-2 expression in a chronic inflammatory model, the present study establishes a possibility for a beneficial treatment targeting EP4, and further sustains the use of intravesical BoNT-A in the treatment of bladder inflammatory diseases [5].

The results of this study increase our actual knowledge on the mechanisms controlling micturition and pain, and stimulate further applications on this field of research. Furthermore, they confirm that the activity of the neurotoxin is not limited to the mere block of the neuromuscular junction. The authors have my personal compliments for their great intuition and for their elegant work.

References


DOI: 10.1016/j.eururo.2008.05.009
DOI of original article: 10.1016/j.eururo.2008.05.007