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ORIGINAL PAPER

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Botulinum toxin type A may improve bladder function in a rat chemical cystitis model

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Abstract The purpose of this study was to investigate the effect of botulinum toxin type A on bladder function and histology in a rat chemical cystitis model. The study included 41 female Sprague-Dawley rats with chemical cystitis induced by intravesical instillation of hydrochloric acid. The acid instillation was repeated monthly to maintain chronic inflammation. The treatment group ($n=21$) received 2–3 units of botulinum toxin type A injected into the bladder detrusor at the 3, 6, 9 and 12 o'clock positions, and the control group ($n=20$) underwent saline injection into the bladder detrusor at the same positions. Urodynamic studies were performed in all rats before the treatment and at death. The rats were killed at 1 week, 2 weeks, 1 month and 2 months after treatment. The bladders were removed and examined histologically for mast cells and inflammatory changes. The cystometric findings showed that, at the beginning and end of the experiment, the increases in the maximum bladder capacity and compliance were significantly higher in the treatment group than in the control group ($P=0.000$ and $P=0.025$, respectively). The histological studies revealed similar mast cell counts and leukocyte infiltration for the treatment and control groups. In conclusion, in this rat chemical cystitis model, botulinum toxin type A injected into the bladder detrusor led to a functional improvement. Thus, botulinum toxin type A injection may be an alternative, minimally invasive choice to other surgical treatment options in the treatment of a chronic inflammatory condition to improve deteriorated bladder function.

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Introduction

Chronic inflammatory disease of the urinary bladder is generally characterized by frequent of urination, urgency, and pelvic pain with bladder distension. Interstitial cystitis is one of the most commonly seen chronic, inflammatory diseases of the urinary bladder [9]. Although several theories have been proposed to explain the pathogenesis of interstitial cystitis, this remains unknown [16].

Severe chronic inflammation of the bladder is associated with reduced bladder capacity and compliance. Although the precise cellular mechanisms of impaired bladder capacity and compliance remain undefined [12], clinical experience has demonstrated that poor bladder capacity and compliance can be dramatically improved by surgical augmentation of bladder volume utilizing intestinal segments, or by anticholinergic drug therapy to decrease detrusor tonicity [3, 10, 13]. The goal of both treatments is the creation and/or preservation of low pressure bladder storage function with increased bladder capacity. However, the high incidence of systemic anticholinergic side effects [22] and short- and long-term complications with surgical treatment may limit long-term patient compliance [2, 23]. Thus, we hypothesized that botulinum toxin type A injected into the bladder detrusor might be an attractive alternative to improve deteriorated bladder capacity and compliance in a diseased bladder.

Botulinum toxin type A selectively blocks acetylcholine release at the presynaptic neuromuscular junction [24]. Inhibited acetylcholine release causes decreased muscle contractility and muscle atrophy at the site of injection. Botulinum toxin type A has been used to treat a spectrum of neuromuscular and neuro-urological diseases [1, 14, 20]. However, to our knowledge, no study

has been carried out on the use of botulinum toxin in the treatment of chronic cystitis such as interstitial cystitis.

The purpose of this study was to investigate the effect of botulinum toxin type A on bladder function in a rat chemical cystitis model. We also histologically investigated the effects of botulinum toxin type A injection into the detrusor on chronically inflamed bladder in rats.

Materials and methods

Animals and study design

A total of 48 female Sprague-Dawley rats, 200–300 g, were used of which seven died during the experiments. Therefore, the study included data from 41 rats: 21 in the treatment group and 20 in the control group. In all rats, intravesical instillation of HCl induced chemical cystitis. This treatment was repeated monthly in order to maintain the chronic inflammation. One week after HCl instillation, all rats were examined urodynamically to determine the baseline bladder pressure, capacity and compliance before the treatment. After performing the baseline urodynamic studies, the rats in the treatment group received 2–3 units of botulinum toxin type A injected into the detrusor at the 3, 6, 9 and 12 o'clock positions, and the rats in the control group underwent the same procedure using saline. Urodynamic examinations were repeated in all rats at death. The rats were killed at 1 week (six treated, five control), 2 weeks (five treated, five control), 1 month (five treated, six control) and 2 months (five treated, four control). The bladders were removed and examined histologically for mast cells and inflammatory changes.

Induction of chemical cystitis

Chemical cystitis was induced by the intravesical instillation of hydrochloric acid (0.2 ml of 0.4 N HCl), as described by Rivas et al. [17]. Rats were anesthetized with ketamine (100 mg/kg) and, under sterile conditions, a 22 gauge catheter was inserted transurethrally. After all of the urine was aspirated, HCl was instilled into the bladder lumen for 4 min. On insertion of the catheter, the rats received ciprofloxacin (20 mg/kg i.m.) to prevent urinary infection. Therefore, the rats in the 1 week and 2 week groups received intravesical instillation of HCl once, the rats in the 1 month group twice, and the rats in the 2 months group three times.

Treatment protocol

At 1 week after the initial HCl instillation, the rats were anesthetized with ketamine (100 mg/kg). They then received a single 20-mg/kg dose of ciprofloxacin. After performing a urodynamic study, the urinary bladder was exposed via a small suprapubic incision under sterile conditions. Two to three units (0.2–0.3 ml.) of botulinum toxin type A (Botox, Allergan, Irvine, Calif., USA) were injected into the detrusor at the 3, 6, 9 and 12 o'clock positions (10–12 sites) in the rats of the treatment groups. The same dose of normal saline was injected into the detrusor at the same positions (10–12 sites) in the rats of the control groups. The rectus fascia was closed with a running 4–0 plain absorbable catgut suture, and the skin was closed with interrupted 3–0 silk sutures.

Functional evaluation

Urodynamic studies were performed before the treatment and at death, as previously described [6]. All rats underwent cystometry under ketamine anesthesia (100 mg/kg i.m.). A 22 gauge catheter was inserted transurethrally and connected by a polyethylene tube to a pressure transducer using urodynamic equipment (Life-Tech, Houston, Tex.) and computer software (Urolab Primolus). After the measurement of the residual urine volume, each rat underwent cystometric measurements with the infusion of warmed (37°C) normal saline solution at 0.1 ml/min (Abbott infusion pump). During the study, the baseline pressure (empty bladder), opening pressure (at first leakage) and peak pressure (maximum pressure during voiding) and the maximum bladder capacity were recorded. Bladder compliance (ml/cm H_20) was calculated according to the following formula [6, 15]: compliance = maximum bladder volume/(opening pressure–baseline pressure).

Histological evaluation

The bladder was removed through a lower midline abdominal incision. After removal, the rats were killed by pentobarbital overdose (200 mg/kg) and bilateral thoracotomy. After both ureters were ligated, the bladder was filled with 10% formalin solution through a catheter transurethrally and kept distended overnight. The specimen was then split longitudinally, and routine tissue processing for light microscopy was performed.

Bladder tissues were embedded in paraffin. Sections (4 μm) were cut by microtome and stained with hematoxylin-eosin (H and E) to assess inflammatory changes with the number of leukocytes, and toluidine blue for mast cells. Slides were examined by an Olympus BX50 light microscope and photographed with an Olympus PM10SP photographic system.

Leukocyte infiltration was evaluated to determine the severity of the inflammation that resulted from intravesical instillation [4]. Each section was divided into ten subsections and leukocytic infiltration was examined in each of the subsections at a magnification of 400× with the following scale: 0: no extravascular leukocytes; 1: < 20 leukocytes/HPF; 2: 20–45 leukocytes/HPF; 3: > 45 leukocytes/HPF. The total score for all subsections was divided by the maximum possible score (30 in ten subsections of the section) and multiplied by 100.

The total number of mast cells was counted at a magnification of 200× in ten random sections of the bladder from each rat. The average number of mast cells was used for comparison.

Statistical analysis

Statistical analyses were performed using the Student's *t*-test for independent data to compare differences between the treatment and control groups in cystometric findings at the beginning and end of each experiment, body weights, degree of leukocytic infiltration and number of mast cells. Data are presented as mean ± SD for the histological findings and as mean ± SE for the cystometric study findings. Probability values of $P < 0.05$ were considered to be statistically significant.

Results

Morbidity and mortality

Of the initial 48 rats, 24 were in the treatment group and 24 were in the control group. Of the seven rats that died during the experiment and were excluded from the study, 3 were in the treatment group and 4 were in the control group. In both treatment and control groups, two rats died from respiratory failure within 24 h due to either anesthesia or HCl instillation. The other one treatment and two control rats died due to either surgery or anesthesia within 1 week of treatment.

Macroscopically slight hematuria was observed in all rats after the HCl instillation which typically disappeared within 1 week. No morbidity (acute or chronic) or mortality was observed after botulinum toxin type A injection into the detrusor in the rats.

After bladder removal, discrete petechiae and/or localized edematous changes in the bladder were observed grossly in ten (47.6%) of the 21 rats in the treatment group and in ten (50%) of the rats in the control group. Bladder stone was found in one (4.7%) of the 21 rats in the treatment group and in one (5%) of the 20 rats in the control group. Metabolic analysis revealed struvite stone in both.

Functional study findings

No significant differences in body weight were observed between the treatment and control groups at any time ($P=0.709$ for 1 week, $P=0.869$ for 2 weeks, $P=0.365$ for 1 month, and $P=0.232$ for 2 months).

No significant differences were noted at the beginning and end of the experiment in the residual urine volume, baseline pressure and maximum voiding pressure between the treatment and control groups ($P=0.192$, $P=0.955$ and $P=0.452$, respectively). However, the mean maximum bladder capacity increased from 1.57 ± 0.15 ml to 2.2 ± 0.23 ml in the treatment groups, and decreased from 1.65 ± 0.22 ml to 1.26 ± 0.13 ml in the control groups. The difference in the increase or decrease of the mean maximum bladder capacity was highly significant between the two groups ($P=0.000$). The mean bladder compliance increased from 0.039 ± 0.005 ml/cm H_2O to 0.049 ± 0.007 ml/cm H_2O in the treatment groups, and decreased from 0.038 ± 0.005 ml/cm H_2O to 0.027 ± 0.004 ml/cm H_2O in the control groups, revealing a significant difference between the two groups ($P=0.025$).

Figures 1 and 2 show the mean maximum bladder capacity and bladder compliance at the beginning and end of the experiment in the treatment and control groups. At 1 week, the mean maximum bladder capacity increased by $45.5\%\pm30.2$ in the treatment group and decreased by $25.7\%\pm13.6$ in the control group. The difference was significant ($P=0.051$). At 2 weeks, the mean maximum bladder capacity increased by $88\%\pm33.7$ in the treatment group and decreased by $42.2\%\pm4.4$ in the control group. This difference was highly significant ($P=0.003$). At 1 month, the mean maximum bladder capacity increased by $19.1\%\pm15$ in the treatment group and decreased by $3\%\pm1.6$ in the control group ($P=0.489$). At 2 months, the increases in the mean maximum bladder capacity were $62.4\%\pm44.5$ and $13.6\%\pm34.6$, respectively ($P=0.436$).

At 1 week, the mean bladder compliance increased by $44.6\%\pm40.8$ in the treatment group and decreased by $13.9\%\pm18.2$ in the control group ($P=0.254$). At 2 weeks, the mean bladder compliance increased by $56.8\%\pm48.1$ in the treatment group and decreased by $55.1\%\pm5.6$ in the control group, revealing a significant difference between the two groups ($P=0.014$). At 1 month, the decrease in the mean bladder compliance was $23.4\%\pm54$ in the treatment group and $5\%\pm17.6$ in the control group ($P=0.755$). At 2 months, the increase in the mean bladder compliance was $71.6\%\pm60.6$ and $14\%\pm35.9$, respectively ($P=0.472$).

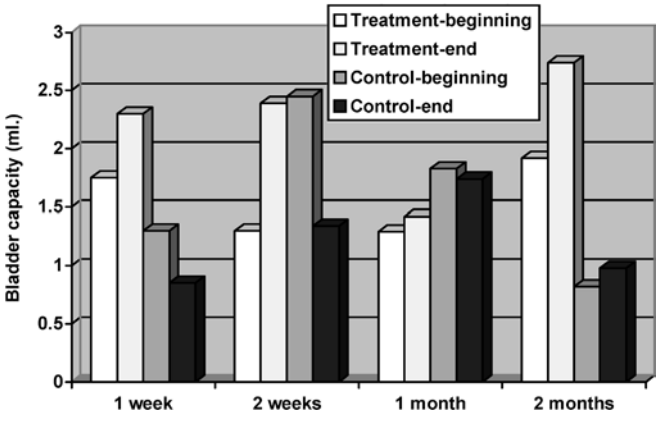

Fig. 1 The maximum bladder capacity at the beginning and end of the study for both treatment and control groups. Note that significant differences in the increases in maximum bladder capacity from the beginning to the end of the study between the treatment and control groups at 1 week and 2 weeks ($P=0.051$ and $P=0.003$, respectively)

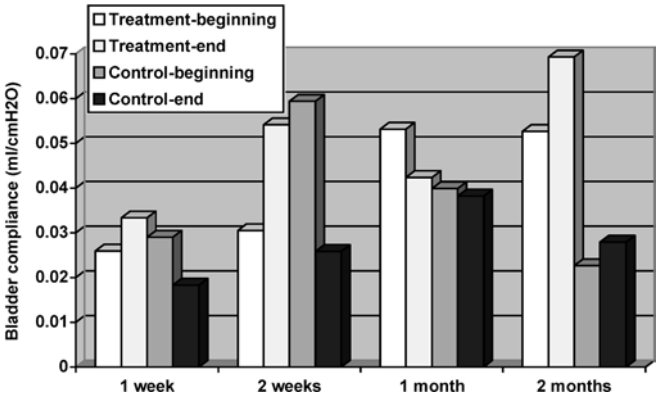

Fig. 2 The bladder compliance at the beginning and end of the study for both treatment and control groups. Note that significant differences in the increases of bladder compliance from the beginning to the end of the study between the treatment and control groups at 2 weeks ($P=0.014$)

Histological findings

Figure 3 shows the number of mast cells in the treatment and control groups. No significant differences were observed in the mean number of mast cells between the treatment and control groups ($P=0.127$ for 1 week, $P=0.109$ for 2 weeks, $P=0.344$ for 1 month, and $P=0.466$ for 2 months). At 1 week, the number of mast cells in the bladders was higher in the control group than

in the treatment group, but this difference was not statistically significant (Fig. 4). Figure 4A and B show mast cells in each bladder of the treatment and control groups at 1 week.

Figure 5 shows the degree of leukocyte infiltration in the treatment and control groups. No significant differences were observed in the degree of leukocyte infiltration between the two groups ($P = 0.732$ for 1 week, $P = 0.683$ for 2 weeks, $P = 0.250$ for 1 month, and $P = 0.213$ for 2 months). As shown in Fig. 6A and B, leukocyte infiltration was similar in all of the bladders from the treatment and control groups at 1 month.

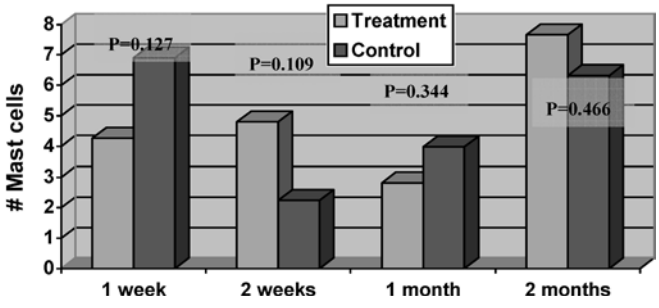

Fig. 3 Mast cell numbers in the treatment and control groups. Note that there are no significant differences in the mean number of mast cells between the treatment and control groups ($P = 0.127$ for 1 week, $P = 0.109$ for 2 weeks, $P = 0.344$ for 1 month, and $P = 0.466$ for 2 months)

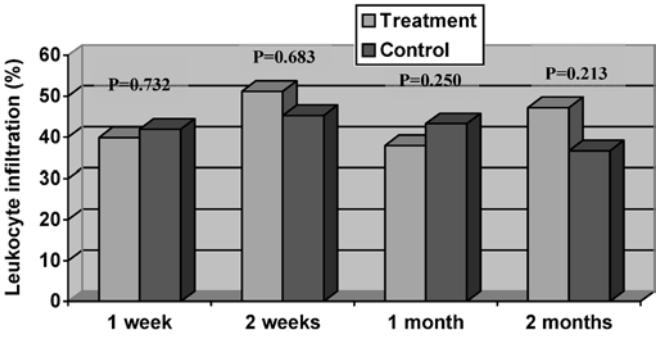

Fig. 5 The degree of leukocyte infiltration in the treatment and control groups. There are no significant differences in the degree of leukocyte infiltration between groups ($P = 0.732$ for 1 week, $P = 0.683$ for 2 weeks, $P = 0.250$ for 1 month, and $P = 0.213$ for 2 months)

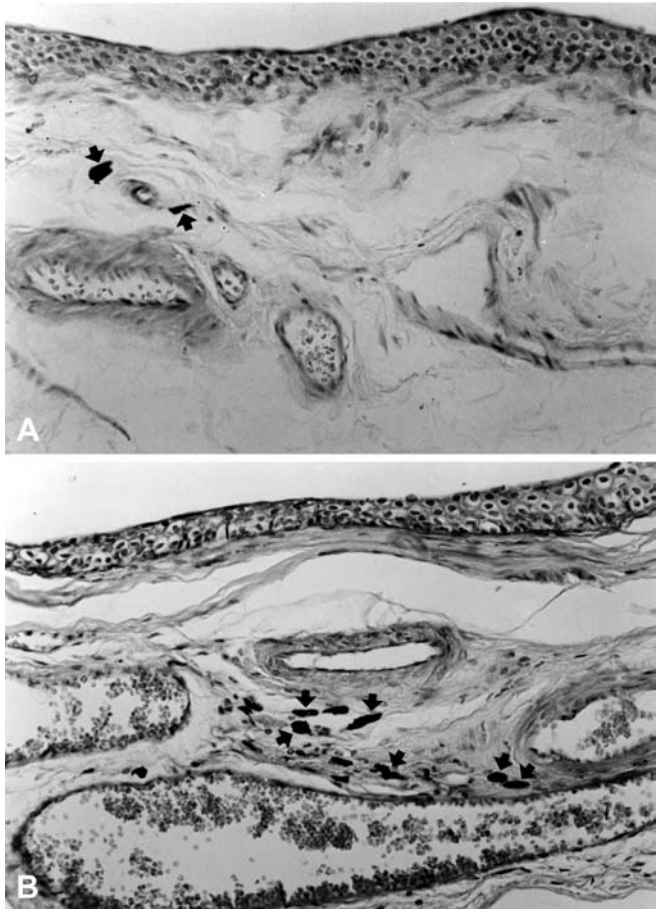

Fig. 4 Toluidine blue staining to show mast cells (*arrows*) (200×). **A** Mast cells in the bladder of a rat in the treatment group at 1 week. **B** Mast cells in the bladder of a rat in the control group at 1 week

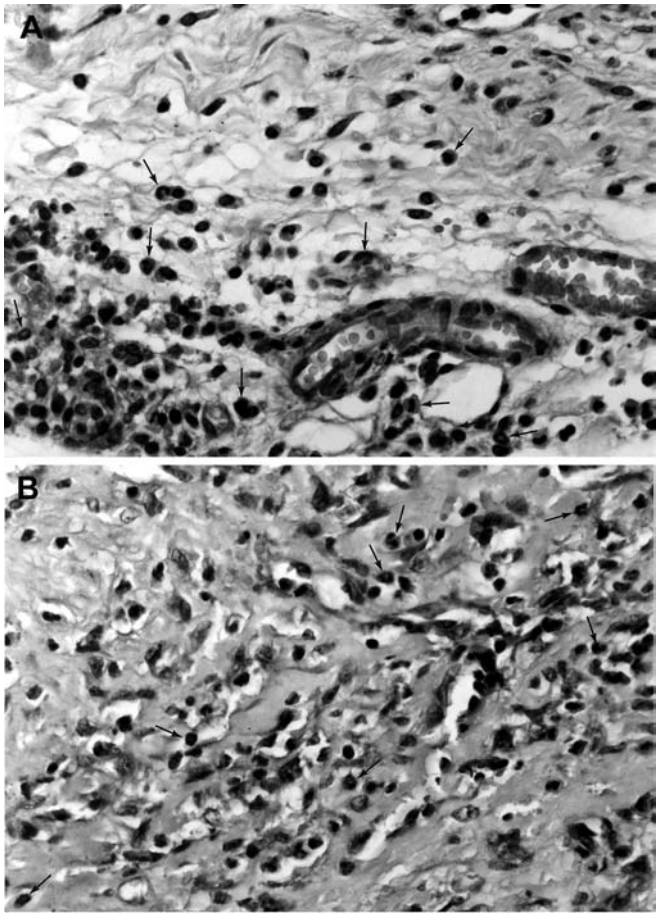

Fig. 6 Histological appearance of the bladder in a rat of the treatment group **A** and control group **B** with hematoxylin-eosin staining (400×) at 1 month. *Arrows* show leukocytic infiltration

Discussion

Current treatment options for a bladder with decreased capacity and/or compliance are either intravesical or oral use of anticholinergic agents [3, 8, 13]. When the treatment is not sufficiently effective, the surgical options are either auto-augmentation or augmentation with detubularized gastrointestinal segments, which can be used to improve deteriorated bladder capacity and compliance [2, 10, 23]. However, the common systemic side effects of the anticholinergic treatment and the short- and long-term complications of the surgical options led urologists to investigate new treatment modalities [2, 22, 23]. In the present study, we investigated the effect of botulinum toxin type A injected into the bladder detrusor on bladder function and histology in a rat chemical cystitis model. This is a minimally invasive option compared to other surgical treatments.

Several animal models have been developed to stimulate inflammation of the urinary bladder, e.g., interstitial cystitis. However, most of the studies were not designed to evaluate the inflammatory reaction in a chronic model. The intravesical instillation of HCl or other acids induces a sustained inflammatory response within the bladder wall for up to one month [7, 17]. We injected botulinum toxin type A into the detrusor 1 week after intravesical HCl instillation because the inflammation remains strong for at least 2 weeks. Rivas et al. also suggested that repeated HCl instillation might create chronic inflammation, although they did not specify the frequency [17]. Therefore, we repeated HCl instillation monthly to maintain chronic inflammation within the bladder.

Botulinum toxin type A is a selective inhibitor of acetylcholine release at the presynaptic neuromuscular junction [24]. Inhibited acetylcholine release causes decreased muscle contractility and muscle atrophy at the site of injection. Botulinum toxin type A has been successfully used for the treatment of a spectrum of neuromuscular and neuro-urological diseases [1, 11, 14, 18, 20, 21, 26]. However, to our knowledge, no study has reported the use of botulinum toxin in the treatment of chronic inflammatory diseases such as chronic cystitis and interstitial cystitis.

Zermann et al. used a perisphincteric injection of botulinum toxin type A in the management of chronic prostatic pain [26]. They reported pain relief and symptom improvement with no immediate or delayed systemic side effects. Schurch et al. evaluated the efficacy of botulinum toxin injected into the detrusor muscle in the patients with traumatic spinal cord injury [20]. They found that the overall mean reflex volume and mean maximum cystometric bladder capacity significantly increased after treatment, with no side effects. Phelan et al. reported a botulinum toxin urethral sphincter injection to restore bladder emptying in men and women with voiding dysfunction including neurogenic detrusor-sphincter dyssynergia, pelvic floor spasticity and acontractile detrusor [14]. They found that 67% of the patients had significant, subjective improvement in voiding. They also reported that the postoperative post-voiding residual volume decreased by 71% and voiding pressures decreased on average 38%. In our experimental study, botulinum toxin type A injections did not affect post-voiding residual volume, baseline pressure, bladder opening pressure or maximum voiding pressure at the beginning or end of the experiment. However, in all rats, the increases in the mean maximum bladder capacity and bladder compliance were significantly higher in the treatment groups than in the control groups.

Mast cells have been closely related to chronic inflammation of the urinary bladder [4, 19]. Their presence is not pathognomonic, but their activation has been associated with the disease. Mast cells also have a neuroimmunomodulatory effect in interstitial cystitis [25]. In the present study, the mast cell count was not significantly different at any time between the treatment and control groups. Mast cells release cytokines, kinins, histamine, leukotrienes, prostaglandins, and proteases. In addition, in the present study, leukocyte infiltration after treatment with botulinum toxin or saline was similar between the treatment and control groups. Thus, our study showed that botulinum toxin type A injection did not provide an histological improvement, such as less mast cells or leukocyte infiltration in the diseased bladders.

Botulinum toxin type A is a highly selective and safe neurotoxin [26]. It does not penetrate the blood-brain barrier at the doses used therapeutically. No significant adverse effect of botulinum toxin injections on the central nervous system has been reported. In our study, we did not observe any acute or chronic complications that could be associated with botulinum toxin injected into the bladder detrusor in rats. Our results are consistent with the clinical studies [14, 20, 26] on the use of botulinum toxin injected into the detrusor or sphincter in humans.

In clinical use, the main disadvantages of injections with botulinum toxin would be repeated cystoscopy and the number of injections that would be necessary to maintain clinical improvement. The chemical denervation provided by botulinum toxin is a reversible process, as new axons re-sprout in 3–6 months [5]. The duration of the toxin effect has been reported to be approximately 3–9 months for a single injection in the treatment of detrusor sphincter dyssynergia [14, 20]. In our study, in the treatment groups, the effect of botulinum toxin type A continued with time. However, the increase in the maximum bladder capacity reached statistical significance at 1 week and 2 weeks in the treatment groups when compared to the control groups. This could be explained if the toxin achieved the highest improvement at 1 week and 2 weeks, and the effect decreased with time at 1 month and 2 months in the diseased bladders. Therefore, our study suggest that repeated injections of the toxin are needed to maintain clinical improvement.

In conclusion, in this rat chemical cystitis model, botulinum toxin type A injected into the bladder detrusor provided functional improvement with no side effects in the diseased bladder. Thus, botulinum toxin type A injection may be an alternative, minimally invasive choice to improve deteriorated bladder function compared to other surgical treatment options for chronic, inflammatory bladder conditions .

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