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Original Article

Intraprostatic Botulinum Neurotoxin Type A Injection for Benign Prostatic Hyperplasia: Preliminary Results with a Newly Purified Neurotoxin

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Several studies have demonstrated the efficacy of intraprostatic injection of botulinum neurotoxin type A (BoNT/A) against symptomatic benign prostatic hyperplasia (BPH). The most commonly used BoNT/A product, Botox®, forms large complexes and composed of neurotoxin (NTX) as well as non-toxic components. We purified NTX lacking non-toxic components. We investigated the efficacy of this newly purified NTX for men with BPH. Ten male patients (mean age, 70.0 years) with BPH received 100 units (prostate volume [PV] <30 ml) or 200 units (PV ≥ 30 ml) of NTX injected into the prostate via a minimally invasive outpatient technique. Evaluation included uroflowmetry, postvoid residual urine volume (PVR), PV, and International Prostate Symptom Score (IPSS) measured at baseline and 1, 3, 6, and 12 months post-treatment. The status of 7 of the 10 patients examined was found to have improved within 1 month of treatment. The mean IPSS decreased from 23.8 ± 7.0 to 16.3 ± 10.3 ($p = 0.0093$) at 1 month, to 14.9 ± 8.2 ($p = 0.0074$) at 3 months, and to 16.9 ± 7.3 ($p = 0.018$) at 12 months. The mean PV decreased from 47.8 ± 21.2 to 39.2 ± 19.5 ml ($p = 0.0076$) at 3 months. The PVR improved at 3 and 6 months post-treatment. Intraprostatic NTX injection induces prostate shrinkage and is effective in men with BPH.

Key words: botulinum neurotoxin type A, benign prostatic hyperplasia, therapy

Benign prostatic hyperplasia (BPH) is a chronic condition associated with lower urinary tract symptoms (LUTS). Therapeutic options for LUTS associated with BPH have expanded rapidly in the last 2 decades. Treatment for BPH aims to relieve 2 types of urinary tract obstruction: mechanical urinary tract obstruction caused by tissue compression due to an enlarged prostate, and functional urinary tract obstruction caused by constriction of the urinary tract and prostatic smooth muscle via sympathetic α1 adrenoceptors (α1-AR). As a result, α1-AR blockers are widely recognized as the first-line pharmacotherapy for BPH treatment [1]. Transurethral resection of the prostate remains the gold-standard surgical treatment for BPH, although this approach is being challenged by new minimally invasive treatments such as laser interventions. The goals of therapy are to improve LUTS and minimize any adverse events of treatment.

Since clinical botulinum neurotoxin type A (BoNT/A) investigations into treatments for BPH started in 2003 [2], several studies have demonstrated the efficacy of intraprostatic BoNT/A injection therapy for patients with BPH [3–8]. The

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results of these studies have demonstrated that BoNT/A injection induced atrophy and diffuse apoptosis of the prostate gland and that the effect persisted for at least 12 months without any notable side effects [9].

There are several commercially available BoNT/A products worldwide. Botox® and Dysport® are the most commonly used BoNT/A products available for urological use [10]. BoNT/A preparations of Botox® and Dysport® are progenitor neurotoxins (PTXs), which consist of both toxic and non-toxic components. The PTXs are found in 3 forms with molecular masses of 900 kDa (19S), 500 kDa (16S), and 300 kDa (12S). The PTXs dissociate into a neurotoxin (NTX) and a non-toxic component under alkaline conditions [11]. As they are easily obtained and are more stable than NTXs, PTXs are in clinical use. However, these PTXs induced side effects in some patients in whom anti-PTX, including anti-NTX antibodies, are produced after the administration of several injections. Recently, a pure NTX (Xeomin®) that is free from complexing proteins was made commercially available; the clinical effectiveness of this product has been demonstrated [12, 13]. Using a simple procedure, our group also purified NTX lacking non-toxic components, and we previously reported its clinical efficacy in refractory urge incontinence patients [14]. In this study, we investigated the effects of this newly purified NTX in patients with symptomatic BPH.

Materials and Methods

Preparation of neurotoxin (NTX). NTX was obtained according to the following straightforward method. After PTX consisting of toxic and non-toxic components in 10 mM sodium phosphate buffer (pH 6.0) was applied to a lactose gel column equilibrated with the same buffer (hemagglutinin-positive toxins bind to the column), the pH of the column was then changed using 100 mM sodium phosphate buffer (pH 8.0). Due to this change in pH, the NTX dissociated from the non-toxic components on the column. A non-toxic component-rich preparation was then eluted with a 10-mM sodium phosphate buffer (pH 6.0) containing 0.05 M lactose [11].

We examined the mutagenicity of NTX and the existence of prion protein and endotoxin prior to conducting this human trial. A mutagenicity assay (AMES test) of NTX (1,000 or 10,000 minimal lethal dose/ml) and albumin (50 mg/ml) was performed according to the preincubation technique of Yahagi and colleagues [15]. Contamination of the NTX preparations (300 μg/ml), the hemagglutinin-positive progenitor neurotoxin (19S and 16S; 0.3, 1, 3 mg/ml), and albumin (0.5, 5 mg/ml) with prion protein was assayed using a Prionics-Check Western kit (Prionics AG, Schlieren, Switzerland). The NTX was filtered with a 0.22-μm-pore-size membrane and then diluted to 100,000 minimal lethal dose/ml in isotonic sodium chloride solution. Contamination with endotoxin was determined by a Limulus amoebocyte lysate assay (BioWhittaker, Walkersville, MD, USA) [16, 17].

Human trial. The effects of NTX on the human prostate were evaluated in 10 men with symptomatic BPH who showed inadequate responses to α1-AR blockers. The study was approved by the institutional review board of Kawasaki Medical School Hospital and written informed consent was obtained from all participants.

Ten patients, aged 60 to 79 years old (median age, 70), were treated between October 2006 and November 2007. The patients had a complete urological assessment before treatment, which included measurement of serum prostatic specific antigen (PSA) level, transrectal ultrasonography, and urine analysis. All patients had an International Prostate Symptom Score (IPSS) of $\geq$8 and a maximum urinary flow rate (Qmax) of $\leq$12 ml/s. At the time of enrollment, all patients had stopped any prescription BPH medical therapy including α1-AR blockers, and no BPH-associated medical therapy was prescribed thereafter. All procedures were performed in the lithotomy position, with a transperineal (6 men) or transrectal (4 men) approach under the guidance of transrectal ultrasonography, and the procedure was carried out under a caudal block (1% lidocaine 10 ml).

NTX at 100 U (2 men, for a prostate volume [PV] of $<$30 ml) or 200 U (8 men, for a PV of $\geq$ 30 ml) was injected into the prostate. The 100 U or 200 U dose of NTX was dissolved in 4 ml of saline immediately before treatment. A 20-cm needle (21-G) was used, and 2 injections of an equal volume (2 ml) were administered into each lobe of the gland. The patients were sent home without a catheter and were instructed to take 300 mg of levofloxacin orally for 2 days.

The evaluations included clinical determination of the IPSS, quality of life (QOL) index, maximum urinary flow rate (Qmax), postvoid residual urine volume (PVR) detected by ultrasonography, and PV before treatment and 1 week, 1, 3, 6, 9, and 12 months post-treatment. The PSA level was measured before treatment, and 6 and 12 months post-treatment.

Statistical analysis. Values are reported as the mean±standard deviation (SD). The Wilcoxon signed rank test was used, and a p-value of less than 0.05 was considered significant.

Results

At 1 week post-injection, the mean IPSS and QOL index were significantly improved (Table 1). Four of 10 patients reported an improvement in symptoms starting within 7 days post-NTX treatment. Seven of the 10 patients noted an improvement within 1 month and were satisfied with this treatment. Three patients were considered to have unchanged symptoms during the follow-up period.

The mean IPSS (for both storage and voiding symptoms) and QOL index significantly improved from 1 week to 12 months. The mean IPSS decreased from 23.8 ± 7.0 to 16.3 ± 10.3 ($p = 0.0093$) at 1 month, to 14.9 ± 8.2 ($p = 0.0074$) at 3 months, to 13.8 ± 7.5 ($p = 0.0076$) at 6 months, and to 16.9 ± 7.3 ($p = 0.018$) at 12 months. The maximum effect of the IPSS, namely, a 42.0% reduction, was seen during the period from 6 to 9 months. However, the effect gradually decreased at 12 months. The IPSS voiding symptoms significantly improved from 1 month to 12 months post-injection. The IPSS storage symptoms significantly improved from 3 months to 12 months. The maximum effects for voiding and storage symptoms were 42.4% and 49.4% reductions from baseline, respectively. The Qmax value significantly improved only at 3 months, and PVR also improved at 3 and 6 months post-treatment (Table 1). The maximum improvement in Qmax was 39.7% from 6.3 ml/s at baseline to 8.8 ml/s at 3 months ($p = 0.0367$). The maximum reduction in PVR was 46.8% from 99.5 ml at baseline to 52.9 ml at 6 months ($p = 0.0152$). The PV decreased 18.0% from 47.8 ml at baseline to 39.2 ml at 3 months post-treatment ($p = 0.0076$). The mean total serum PSA decreased from 4.30 ng/ml at baseline to 3.75 ng/ml at 6 months, which corresponded to a 12.8% reduction, however, this decrease was not

Table 1 Patient profiles and results after NTX treatment

	Number of patients	IPSS	QOL	Qmax (ml/s)	PVR (ml)	PV (ml)	PSA (ng/ml)
		Storage, Voiding					
Baseline	10	23.8 ± 7.0 8.7 ± 4.8, 11.8 ± 3.4	5.2 ± 1.0	6.3 ± 3.1	99.5 ± 94.5	47.8 ± 21.2	4.30 ± 3.0
1 week	10	$19.4 \pm 9.3^*$ 7.2 ± 4.5, 9.5 ± 4.4	$4.3 \pm 1.6^*$	5.5 ± 2.0	84.9 ± 83.1	45.9 ± 22.3	NA
1 month	10	$16.3 \pm 10.3^*$ 6.7 ± 5.4, $6.9 \pm 5.0^*$	$3.4 \pm 1.6^*$	6.1 ± 2.6	101.5 ± 97.0	$40.2 \pm 18.2^*$	NA
3 months	10	$14.9 \pm 8.2^*$ $5.3 \pm 3.8^*$, $7.4 \pm 4.8^*$	$3.3 \pm 1.8^*$	$8.8 \pm 2.9^*$	$65.3 \pm 73.6^*$	$39.2 \pm 19.5^*$	NA
6 months	9	$13.8 \pm 7.5^*$ $4.4 \pm 3.5^*$, $7.0 \pm 4.2^*$	$3.2 \pm 1.6^*$	6.8 ± 3.9	$52.9 \pm 62.5^*$	$40.2 \pm 19.2^*$	3.75 ± 2.2
9 months	9	$13.8 \pm 7.6^*$ $5.4 \pm 3.5^*$, $6.8 \pm 4.5^*$	$3.7 \pm 1.4^*$	6.2 ± 2.2	86.3 ± 100	42.9 ± 23.2	NA
12 months	8	$16.9 \pm 7.3^*$ $5.2 \pm 4.3^*$, $7.4 \pm 4.6^*$	$4.3 \pm 1.5^*$	7.2 ± 4.0	72.4 ± 58.2	41.0 ± 17.0	4.35 ± 2.8

NA: not available, *Compared with the baseline, $p < 0.05$

IPSS, International Prostate Symptom Score; QOL, quality of life; Qmax, maximum urinary flow rate; PVR, postvoid residual urine volume; PV, prostate volume; PSA, prostatic specific antigen.

reach statistically significant.

At a mean follow-up of more than 36 months, 5 of 7 responsive patients reported a worsening of symptoms during the 12 months after treatment. Finally, 5 patients received transurethral holmium laser enucleation of the prostate within 24 months post-treatment. No remarkable changes were observed in the histology of the resected prostatic tissue (data not shown). No systemic side effects were observed in this study. One patient who had received a transrectal injection suffered from prostatitis without fever, and the patient received antibiotics for 1 week.

Discussion

BPH is a highly prevalent, non-malignant enlargement of the prostate related to aging [18]. BPH may cause bladder outlet obstruction via 2 different mechanisms. One mechanism is a static compression of the urethra by the prostatic adenoma, and the other is a dynamic α1-AR-mediated sympathetic stimulation of the prostatic smooth muscle [19]. Prostatic tissue includes both the epithelial and stromal elements of the gland. The epithelial elements are more sensitive to androgen deprivation, and the stromal elements are more sensitive to α1-AR blockers. Some researchers have suggested that prostate growth is controlled in an endocrine manner [20, 21]. There is also some evidence that, while the stromal component of the prostate receives predominantly noradrenergic innervation, the epithelial component is innervated by autonomic nerves, and cholinergic fibers are thought to be responsible for acinar secretion. BoNT/A, which inhibits acetylcholine release at nerve terminals, can suppress the secretory function of acetylcholine on the prostate, and it has been shown to cause apoptosis of epithelial elements and a reduction in prostate weight [22, 23]. Prior to the present clinical study, we had investigated this newly purified NTX, and we demonstrated volume reduction and apoptosis of the rat prostate after injection [24]. In the present study, NTX injection induced a 18% reduction of PV at 3 months.

Initially, it was thought that BoNT/A could only act by inhibition of acetylcholine release at cholinergic neuromuscular junctions. However, the effect was found to extend to the neuroglandular junction. Acetylcholine is not the only neurotransmitter affected by BoNT/A. Smith *et al.* found that BoNT/A injection into the rat proximal urethral sphincter induces marked reductions in labeled norepinephrine after high (20 Hz) electrical field stimulation [25]. The inhibition of norepinephrine has attracted much interest in terms of the modulation of lower urinary tract dysfunction, especially in men with BPH. Moreover, BoNT/A has also been shown to inhibit the release of neuropeptides (*e.g.*, calcitonin gene-related peptide) thought to play a role in overactive bladder conditions such as sensory urgency or chronic prostatitis [26, 27]. For these reasons, the use of BoNT/A has been extended to the treatment of symptomatic BPH. Indeed, Chuang *et al.* reported symptomatic relief after BoNT/A (Botox®) injection in patients without shrinkage of the prostate, which can be explained by the induced relaxation of prostatic smooth muscle [4].

NTX was developed to reduce drug antigenicity by the removal of complexing proteins. However, with a size-reduced PTX component, it could be hypothesized that NTX may more rapidly and more easily diffuse away from the target tissue. If such diffusion differences exist, they would be expected to be associated with an adverse effect profile that would differ from that of PTXs such as Botox®. Although in this study, we did not compare PTX and NTX, our results were almost the same as those of previous studies that used PTX in human subjects [9]. Moreover, there were no significant systemic complications in this study. Commercially available NTX (Xeomin®) does not affect the spread of NTX and it has been demonstrated that Xeomin® is not inferiority to Botox® in patients with cervical dystonia [12, 13].

Several clinical studies have demonstrated that beneficial effects were achieved with BoNT/A therapy (Table 2). Currently, α1-AR blockers remain the first-line therapy for the majority of patients with BPH. The total symptom score following medication is improved by 30 to 45%, and Qmax is improved by 15 to 30% versus baseline value [1]. With BoNT/A intraprostatic treatment, the increase in Qmax (39.7–121%) and the decrease in IPSS (47.6–65.5%) were statistically significant in all studies reported to date. There is a statistically significant reduction in PV, which varies from 13.3% to 68.1% (Table 2). The reduction in PV appears to be more prominent in patients with a larger prostate [2, 5]. Although our

Table 2 Comparison of BoNT/A therapy in terms of IPSS, urodynamic parameters, PV, and PSA with the results of previous studies

Study	Number of improved patients (Number of treated patients)	Improved	Before and after treatment			
		IPSS (%−Δ)	Qmax (ml/s) (%+Δ)	PVR (ml) (%−Δ)	PV (ml) (%−Δ)	PSA (ng/ml) (%−Δ)
Maria *et al*. (2003) [2]	13 (15)	23.2 to 8.0* (65.5)	8.1 to 15.4* (90.1)	126 to 21.0* (83.3)	52.6 to 16.8* (68.1)	3.7 to 1.8* (51.4)
Kuo *et al*. (2005) [3]	10 (10)	NA	7.6 to 11.6 (52.6)	243 to 36.8* (84.9)	65.5 to 49.6* (24.3)	NA
Chuang *et al*. (2005) [4]	16 (16)	18.8 to 9.0* (52.1)	7.3 to 11.8* (61.6)	67.7 to 26.8 (60.4)	19.6 to 17.0* (13.3)	0.8 to 0.72 (10.0)
Guercini *et al*. (2005) [5]	16 (16)	24 to 9* (62.5)	8.2 to 18.1* (121)	295 to 85 (71.2)	106 to 53* (50.0)	9.5 to 2.5* (73.7)
Chuang *et al*. (2006) [6]	21 100U / 31 (41) / 20 200U	18.7 to 9.8* (47.6) / 19.3 to 9.5* (50.8)	7.9 to 12.0* (51.9) / 7.0 to 10.3* (47.1)	64.2 to 35.7 (44.4) / 161.7 to 45.2* (72.0)	21.1 to 18.0* (14.7) / 54.3 to 46.3* (14.7)	NA / NA
Brisinda *et al*. (2009) [7]	55 (77)	24.1 to 8.7* (63.9)	8.6 to 16.5* (91.9)	92.1 to 40.6* (55.9)	54.1 to 24.3* (55.1)	6.2 to 3.0* (51.6)
Present study	7 (10)	23.8 to 13.8* (42.0)	6.3 to 8.8* (39.7)	99.5 to 52.9* (46.8)	47.8 to 39.2* (18.0)	4.30 to 3.75 (12.8)

NA: not available, *Compared with the baseline, $p < 0.05$.
IPSS, International Prostate Symptom Score; Qmax, maximum urinary flow rate; PVR, postvoid residual urine volume; PV, prostate volume; PSA, prostatic specific antigen.

study demonstrated only an 18% reduction in PV, it was similar to that found by Kuo and Chuang *et al.* [4, 5]. A recent randomized clinical trial demonstrated that BoNT/A (Botox®) treatment did not induce a decrease in prostate size [28]. In our previous animal study, NTX injection induced marked atrophy and diffuse apoptosis of the rat prostate [24]. The rat prostate is primarily composed of epithelium, whereas the human prostate is primarily stroma [21]. The pathology of BPH is heterogenous. The ratio of epithelium to stroma can vary substantially from 1 : 2 to 1 : 5 in the human prostate [29]. The results of BoNT/A effects in the rat prostate cannot be generalized to the human therapeutic arena. BoNT/A treatment of humans may relieve BPH symptoms by affecting sensory nerve pathways rather than by reducing the prostatic size alone.

In previous study, BoNT/A effects appeared during the first week to 1 month post-treatment [3, 5]. In this study, the beneficial results were evident within 1 week post-treatment, and they continued for 12 months. However, 5 of 7 responsive patients complained of a recurrence of LUTS at 12 months. Finally, 5 patients underwent transurethral holmium laser enucleation of the prostate and were very satisfied with the results. In contrast, Silva *et al.* reported 11 of 21 frail elderly patients with refractory urinary retention resumed spontaneous voiding after BoNT/A (Botox®) injection and subjective and objective improvement was limited [8]. The first randomized, placebo-controlled study showed prostatectomy-like results, but these results were suspected as being too good to be true [2, 9]. Thus, the effectiveness of BoNT/A injection may lie between that of medication and prostatectomy. Intraprostatic BoNT/A injection may be a therapeutic option for patients with refractory BPH who do not elect to undergo surgery.

The therapeutic efficacy BoNT/A is related to injection dosage, diffusion of toxin within the prostate, the ratio of epithelium and stromal components, and to detrusor function. In this study, the improvements in subjective symptoms and objective parameters were almost the same as those previously reported (Table 2). Two of 3 non-responders were suspected to be weak detrusors by urodynamic study, and another patient demonstrated typical middle lobe hypertrophy. There is a possibility that some patients will experience less benefit than others from BoNT/A treatment.

The effects of denervation provided by BoNT/A wear off as new axons re-sprout at around 6 months. Chuang *et al.* and Maria *et al.* reported that the treatment effect emerged at 1 month and was sustained for at least 12 months [3, 6]. In the present study, maximum effects were seen from 3 to 9 months, and PV reached a minimum at 3 to 6 months. Silva *et al.* [8] reported that PV decreased gradually to a minimum at 6 months, corresponding to a 40% reduction. From 6 months onwards, a slow re-growth of the glands was observed. Thus, the previous finding by Silva *et al.* [8] was almost the same as that obtained in our study.

The present human study had some limitations. First, it lacked a placebo control group. Second, no comparison was made between the effects of doses of 100 U and 200 U, nor between the effects of transrectal versus transperineal approaches. As the present study was too small for such comparisons to be made, further investigations will be necessary. We are currently planning to examine whether there are any differences in the respective modes of action of PTX and NTX therapies.

In conclusion, our preliminary results demonstrated that newly purified NTX improved the symptoms of BPH-related LUTS to the same extent as that achieved with PTX. A newly purified intraprostatic NTX injection may be a therapeutic option for patients with refractory BPH.

References

1. Djavan B and Marberger M: A meta-analysis on the efficacy and tolerability of alpha 1-adrenoceptor antagonists in patients with lower urinary tract symptoms suggestive of benign prostatic obstruction. Eur Urol (1999) 36: 1–13.
2. Maria G, Brisinda G, Civello IM, Bentivoglio AR, Sganga G and Albanese A: Relief by botulinum toxin of voiding dysfunction due to benign prostatic hyperplasia: results of a randomized, placebo-controlled study. Urology (2003) 62: 259–264.
3. Kuo HC: Prostate botulinum toxin injection-an alternative treatment for benign prostatic obstruction in poor surgical candidates. Urology (2005) 65: 670–674.
4. Chuang YC, Chiang PH, Huang CC, Hsien K, Yoshimura N and Chancellor MB: Botulinum toxin A improves benign prostatic hyperplasia symptoms in patients with small prostates. Urology (2005) 66: 775–779.
5. Guercini F, Giannantoni A, Bard RL, Brisinda G, Cadeddu F, Maria G and Rossi P: Intraprostatic botulinum toxin injection in patients with severe benign prostatic hyperplasia a multicenter feasible study (Abstract). J Urol (2005) 173 (suppl): 376–377.

6. Chuang YC, Chiang PH, Yoshimura N, Miguel FD and Chancellor MB: Sustained beneficial effects of intraprostatic botulinum toxin type A on lower urinary tract symptoms and quality of life in men with benign prostatic hyperplasia. BJU Int (2006) 98: 1033–1037.
7. Brisinda G, Cadeddu F, Vanella S, Mazzeo P, Marniga G and Maria G: Relief by botulinum toxin of lower urinary tract symptoms owing to benign prostatic hyperplasia: early and long-term results. Urology (2009) 73: 90–94.
8. Silva J, Pinto R, Carvalho T, Botelho F, Silva P, Oliveira R, Silva C, Cruz F and Dinis P: Intraprostatic botulinum toxin type A injection in patients with benign prostatic enlargement: duration of the effect of a single treatment. BMC Urology (2009) 9: 9.
9. Oeconomou A, Madersbacher H, Kiss G, Berger TJ, Melekos M and Rehder P: Is botulinum neurotoxin type A (BoNT-A) a novel therapy for lower urinary tract symptoms due to benign prostatic enlargement? A review of the literature. Eur Urol (2008) 54: 765–777.
10. Chuang YC and Chancellor MB: The application of botulinum toxin in the prostate. J Urol (2006) 176: 2375–2382.
11. Oguma K, Inoue K, Fujinaga Y, Yokota K, Watanabe T, Ohyama T, Takeshi K and Inoue K: Structure and function of Clostridium botulinum progenitor toxin. J Toxicol-Toxin Rev (1999) 18: 17–34.
12. Frevert J: Xeomin®: an innovative new botulinum toxin type A. Eur J Neurology (2009) 16 Suppl. 2: 11–13.
13. Dressler D: Routine use of Xeomin® in patients previously treated with Botox®: long term results. Eur J Neurology (2009) 16 (Supp. 2): 2–5.
14. Lee JC, Yokoyama T, Hwang HJ, Arimitsu H, Yamamoto Y, Takigawa T, Takeshi K, Nishikawa A, Kumon K and Oguma K: Clinical application of *Clostridium botulinum* type A neurotoxin purified by a simple procedure for patients with urinary incontinence caused by refractory destrusor overactivity. FEMS Immunol Med Microbiol (2007) 51: 201–211.
15. Yahagi T, Nagao M, Seino Y, Matsushima T and Sugimura T: Mutagenicities of N-nitrosamines on Salmonella. Mutat Res (1977) 48: 121–129.
16. Gao B and Tsan MF: Recombinant human heat shock protein 60 does not induce the release of tumor necrosis factor from murine macrophages. J Biol Chem (2003) 25: 22523–22529.
17. De Haan L, Hearn AR, Rivett AJ and Hirst TR: Enhanced delivery of enogenous peptides into the class I antigen processing and presentation pathway. Infect Immun (2002) 70: 3249–3258.
18. Jacobsen SJ, Girman CJ and Lieber MM: Natural history of benign prostatic hyperplasia. Urology (2001) 58: 5–16.
19. Lepor H, Tang R and Shapiro E: The α-adrenoceptor subtype mediating thetension of human prostatic smooth muscle. Prostate (1993) 22: 301–307.
20. Ventura S, Pennefather JN and Mitchelson F: Cholinergic innervation and function in the prostate gland. Pharmacol Ther (2002) 94: 93–112.
21. Luján M, Páez A, Llanes L, Angulo J and Berenguer A: Role of autonomic innervation in rat prostatic structure maintenance: a morphometric analysis J Urol (1998) 160: 1919–1923.
22. Doggweiler R, Zermann DH, Ishigooka M and Schmidt RA: Botox-induced prostatic involution. Prostate (1998) 37: 44–50.
23. Chuang YC, Huang CC, Kang HY, Chiang PH, Demiguel F, Yoshimura N and Chancellor MB: Novel action of botulinum toxin on the stromal and epithelial components of the prostate gland. J Urol (2006) 175: 1158–1163.
24. Nishiyama Y, Yokoyama T, Tomizawa K, Okamura K, Yamamoto Y, Matsui H, Oguma K, Nagai A and Kumon H: The effects of purified newly developed botulinum neurotoxin type A in the rat prostate. Urology (2009) 74: 436–439.
25. Smith CP, Franks ME, McNeil BK, Ghosh R, de Groat WC, Chancellor MB and Somogyi GT: Effects of botulinum toxin A on the autonomic nervous system of the rat lower urinary tract. J Urol (2003) 169: 1896–1901.
26. Rapp DE, Turk KW, Bales GT and Cook SP: Botulinum toxin type a inhibits calcitonin gene related peptide release from isolated rat bladder. J Urol (2006) 175:1138–1142.
27. Chuang YC, Yoshimura N, Huang CC, Chiang PH and Chancellor MB: Intravesical botulinum toxin A administration produces analgesia against acetic acid induced bladder pain responses in rats. J Urol (2004) 172: 1529–1532.
28. Crawford ED, Hirst K, Kusek JW, Donnell RF, Kaplan SA, McVary KT, Mynderse LA, Roehborn CG, Smith CP and Bruskewitz R: Effects of 100 and 300 units of onabotulinum toxin A on lower urinary tract symptoms of benign prostatic hyperplasia: a phase 2 randomized clinical trial. J Urol (2011) 186: 965–970.
29. Kyprianou N: Doxazosin and terazosin suppress prostate growth by inducing apoptosis: clinical significance. J Urol (2003) 19: 1520–1525.