

PHARMACOLOGY

IS BOTULINUM TOXIN TYPE A INTRAPROSTATIC INJECTIONS REALLY EFFECTIVE IN PATIENTS WITH URINARY RETENTION?

MIŁOSZ JASIŃSKI^{1,2*}, TOMASZ DREWA^{1,2}, JANUSZ TYLOCH³ AND ZBIGNIEW WOLSKI³¹Department of Urology, Oncology Centre, Romanowskiej 2, Bydgoszcz, Poland²Department of Medical Biology, Nicolaus Copernicus University, Karłowicza 24, Bydgoszcz, Poland³Department of General, Oncological and Pediatric Urology, Nicolaus Copernicus University, Curie Skłodowskiej 9, Bydgoszcz, Poland

Abstract: Botulinum toxin type A is used in treatment of bladder hyperactivity and sphincter dyssynergia and was reported to alleviate lower urinary tract symptoms in patients with BPH. Some authors, however, failed to observe in their study apoptosis after BoNTA administration. We conducted an open-label study of BoNTA in men with BPH-related LUTS who were unsuitable for surgery as well as investigation of the effect of the toxin on *in vitro* growth of fibroblasts. In the clinical part, 5 patients aged from 75 to 88, suffering from BPH and UR were treated. Patients were previously disqualified from surgery and had not passed trials without catheters (TWOC). Prostate volume ranged from 38 to 104 mL. Botulinum toxin injection were performed. Each lobe of adenoma was injected with 100 U Botox under sonographic guidance. Prostate volume and TWOC were performed after 6 months. In the *in vitro* part, 3T3 mouse fibroblasts and fibroblasts isolated from human prostate were cultured in the presence of Botox (10, 5 and 1 U/mL) for 24 and 72 h. Cells were detached and counted in Neubauer chamber using trypan blue assay. Cells cultured in medium without botulinum toxin were the control group. Results are presented as the means with standard deviations. The means were compared, $p < 0.05$ was considered statistically significant. No early complications were observed. Prostate volume remained unchanged after six months and patients were unable to void. Number of 3T3 cells after 24 h incubation was 7.12 ± 1.88 , 7.12 ± 0.64 , 6.75 ± 1.28 and $6.88 \pm 0.83 \times 10^4$, after 24 h, 24.00 ± 3.46 , 22.75 ± 3.73 , 23.12 ± 3.46 and $23.88 \pm 2.42 \times 10^4$ after 72 h, for 0, 1, 5 and 10 U/mL botulinum toxin type A concentrations, respectively. Similarly, number of prostate fibroblasts was 7.50 ± 1.20 , 7.12 ± 1.73 , 6.50 ± 1.93 , and $6.25 \pm 1.58 \times 10^4$ after 24 h and 9.62 ± 2.00 , 9.12 ± 1.55 , 9.12 ± 1.73 and $9.75 \pm 2.82 \times 10^4$ after 72 h. In conclusion, Botox had no statistically significant, dose-dependent effect on neither 3T3 nor prostate fibroblasts proliferation. It caused no improvement in UR nor prostate volume reduction.

Keywords: prostate, BPH, botulinum toxin, urinary retention

Botulinum toxin type A (BoNTA) is one of seven neurotoxins produced by *Clostridium botulinum* and one of the most potent biological toxins known (1). It has been widely used in many medical fields for a long time and in last few years its use was extended to treatment of detrusor overactivity and sphincter dyssynergia (2, 3). Recently, BoNTA application in treatment of benign prostate hyperplasia (BPH) has been investigated, following an experiment in which intraprostatic administration of BoNTA induced prostate atrophy in rat (4). Several authors have reported prostate volume reduction as well as alleviation of lower urinary tract symptoms (LUTS) in patients with BPH after botulinum toxin injection (5–11).

The mechanism through which BoNTA reduces prostate volume is, however, still not fully

explained. Apoptosis of both epithelial and stromal cells after BoNTA administration was observed (4, 5). There are, however, some inconsistencies. Kessler et al., for example, failed to observe in their study apoptosis after BoNTA administration (12).

Encouraged by abovementioned results, we conducted an open-label study of BoNTA in men with BPH-related LUTS who were unsuitable for surgery as well as investigation of the effect of the toxin on *in vitro* growth of fibroblasts – cells from stroma, the dominant compartment of human prostate.

MATERIALS AND METHODS

In the clinical part, 5 patients aged from 75 to 88, suffering from BPH and UR were treated. Patients were previously disqualified from surgery

* Corresponding author: e-mail: urologiaonk@co.bydgoszcz.pl

Table 1. Numbers of viable cells in a well after 24 h or 72 h incubation in medium containing 0, 1, 5 or 10 U/mL of BoNTA.

BoNTA concentration → [U/mL]	0	1	5	10
3T3, 24 h	$7.12 \pm 1.88 \times 10^4$	$7.12 \pm 0.64 \times 10^4$	$6.75 \pm 1.28 \times 10^4$	$6.88 \pm 0.83 \times 10^4$
3T3, 72 h	$24.00 \pm 3.46 \times 10^4$	$22.75 \pm 3.73 \times 10^4$	$23.12 \pm 3.46 \times 10^4$	$23.88 \pm 2.42 \times 10^4$
Prostate fibroblasts, 24 h	$7.50 \pm 1.20 \times 10^4$	$7.12 \pm 1.73 \times 10^4$	$6.50 \pm 1.93 \times 10^4$	$6.25 \pm 1.58 \times 10^4$
Prostate fibroblasts, 72 h	$9.62 \pm 2.00 \times 10^4$	$9.12 \pm 1.55 \times 10^4$	$9.12 \pm 1.73 \times 10^4$	$9.75 \pm 2.82 \times 10^4$

Results are presented as the means \pm SD.

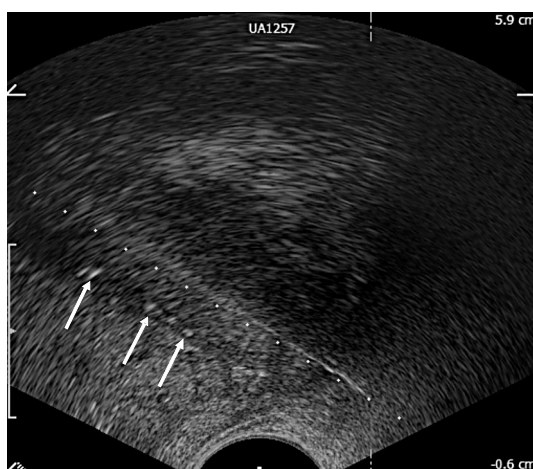


Figure 1. Intraprostatic administration of botulinum toxin under sonographic guidance. Botulinum toxin deposits are indicated with arrows.

and had not passed trials without catheters (TWOC). Prostate volume ranged from 38 to 104 mL, mean 71.2 ± 24.8 mL. Botulinum toxin injections were performed under transrectal sonographic guidance (ProFocus, B&K, Denmark) (Fig. 1). Each lobe of adenoma was injected with 100 U Botox (Allergan, USA) dissolved in 4 mL of saline, without analgesia. Ultrasonographic prostate volume measurement and TWOC were repeated after 6 months. Local ethical committee permission for the study was obtained.

In the *in vitro* part, 3T3 mouse fibroblasts and fibroblasts isolated from human prostate (material from adenomectomy) were used. Cells were seeded in 24-well culture plates with 30,000 cells/well and cultured for 24 h in Dulbecco's Modified Eagle's Medium (Sigma, Germany) with 10% fetal bovine serum, at 37°C, with 5% CO₂. After 24 h, the medium was replaced with fresh and different volumes of

BoNTA solution, which were added to obtain concentrations 10, 5 and 1 U/mL. The culture plates were then incubated at 37°C for 24 or 72 h. Cells were detached, centrifuged and resuspended in PBS. Viable cells were counted using a Neubauer chamber under inverted microscope using trypan blue assay. Cells cultured in the medium without botulinum toxin were the control group. Each experiment was repeated 8 times. Results were presented as the means with standard deviations. The means were compared, $p < 0.05$ was considered statistically significant.

RESULTS

Intraprostatic BoNTA was well tolerated, none of the patients required analgesia and no early complications were observed. At the 6th month evaluation, however, all of the patients were unable to void spontaneously and all of them required catheterization. Prostate volumes did not change significantly – the mean 69.6 ± 23.8 mL, in three patients a decrease and in two an increase of gland volume were observed.

In the *in vitro* part, number of 3T3 cells did not differ from control group neither after 24 h nor 72 h incubation for 1, 5 and 10 U/mL botulinum toxin type A concentrations. Similarly, no significant influence of BoNTA on number of prostate fibroblasts was observed. The results are presented in Table 1 and Figure 2.

DISCUSSION

The reduction of prostate volume after intraprostatic BoNTA administration appears to be connected with widespread apoptosis of both epithelial and stromal compartment of the gland. BoNTA is known to exert an inhibitory effect on somatic and autonomic neurotransmission by inhibiting release of ACh from the nerve into neuromuscular junction

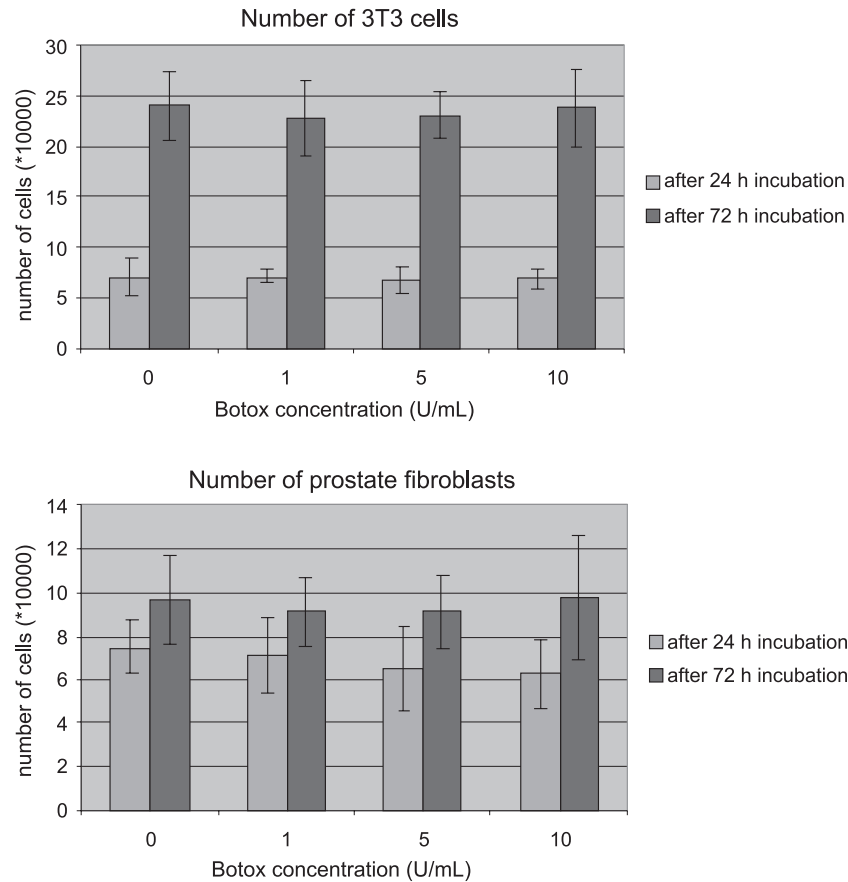


Figure 2. Numbers of viable cells in a well after 24 h or 72 h incubation in medium containing 0, 1, 5 or 10 U/mL of BoNTA, results are presented as the means \pm SD

as well as release of norepinephrine from rat sympathetic fibres (13). This could suggest that the effect of BoNTA on prostate may be connected with the impairment of autonomic innervation. Indeed, Silva et al. demonstrated in an animal model that gland apoptosis induced by intraprostatic BoNTA administration was caused by sympathetic chemodenervation (14).

While cholinergic nerves are more numerous in the acini, adrenergic fibres are located mainly in stroma, the predominant compartment of human prostate (15, 16). While the reduction of volume and apoptosis reported after intraprostatic administration of BoNTA was observed in both epithelial and stroma compartment, the lack of serum PSA level decrease observed in some studies would suggest stronger effect of BoNTA on stroma. That was the rationale to use prostate myofibroblasts in the *in vitro* part.

The lack of response to BoNTA in cultured cells agrees with the proposed and possible mechanism of the toxin effect on prostate gland. The toxin does not induce apoptosis in prostate cells by itself, but rather acts through inhibition of autonomic innervation of the gland.

Since the pioneer study of Maria et al. (6), the influence of BoNTA on prostate in BPH patients was examined in a few other studies (4–11, 17). Most of the authors reported good clinical results and significant reduction of prostate volume. Having considered this, interesting is the fact that we have observed no significant change in prostate volumes after BoNTA administration, while volume reduction of as much as 40% are described in the literature (11). It may be partially attributed to the limited precision of ultrasound prostate volume measurement, the method used in many studies, including this one. This method may have measurement error

up to 20%, even if measurements are performed by the same person.

On the other hand, our clinical results also significantly differ from these of other authors. All our patients were unable to void spontaneously before BoNTA administration and no significant improvement was observed – none of them returned to spontaneous voiding and they all still required catheterization 6 months after treatment.

To conclude, we must state that despite a few optimistic reports in the literature, we failed to observe any significant improvement after BoNTA administration. Further studies of BoNTA effect on human prostate, especially larger, double blind, randomized placebo-controlled studies, should be performed in order to properly evaluate this potential treatment method. Nevertheless, intraprostatic BoNTA administration is a safe procedure as no early nor late complications were observed.

CONCLUSIONS

Botox had no statistically significant, dose-dependent effect on neither 3T3 nor prostate fibroblasts proliferation. It caused no improvement in UR nor prostate volume reduction.

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