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In the Human Urothelium and Suburothelium, Intradetrusor Botulinum Neurotoxin Type A Does Not Induce Apoptosis: Preliminary Results

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Abstract

Background: Intradetrusor injections of botulinum neurotoxin type A (BoNTA) are emerging as the preferred second-line treatment for neurogenic and idiopathic overactive bladder (OAB). In animal experiments, intradetrusor BoNTA injections have been shown to cause apoptosis in the bladder urothelium and suburothelium but not the detrusor.
Objective: To investigate BoNTA-induced apoptosis in patients with refractory neurogenic OAB.
Design, setting, and participants: Twelve refractory OAB patients with neurogenic detrusor overactivity resulting from multiple sclerosis (MS) and seven controls were included prospectively.
Measurements: The number of apoptotic cells before and 4 wk after first intradetrusor BoNTA (300 U of BOTOX [Allergan, Irvine, CA, USA]) injections were estimated using terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) staining.
Results and limitations: Comparison of TUNEL-positive cells (yes vs no) in the bladder urothelium and suburothelium revealed no significant differences in OAB patients before (4 of 12, 33%) versus after (3 of 12, 25%) BoNTA treatment ($p = 0.99$). In addition, no significant differences ($p = 0.99$) were found in OAB patients versus controls. Because our findings are based on first intradetrusor BoNTA injections only, it is unclear whether the results could be extrapolated to repeat injections.
Conclusions: In contrast to preliminary animal experiments, first intradetrusor BoNTA injections for treating refractory neurogenic OAB—a highly effective treatment—did not induce apoptosis in the bladder urothelium and suburothelium.

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1. Introduction

Detrusor injections of botulinum neurotoxin type A (BoNTA) were introduced to treat intractable bladder symptoms resulting from neurogenic detrusor overactivity [1] on the premise that the cholinergic innervation of the detrusor would be blocked [2]. Studies of patients with various causes of spinal cord dysfunction and multiple sclerosis (MS) in particular [3] have demonstrated the efficacy of this intervention [4], and placebo-controlled trials have confirmed a real effect [5,6]. Indeed, its efficacy has been so remarkable that a hypothesis has been proposed that it may not only be acting on the efferent but also on the afferent innervation, because its effect on urgency is so early, profound, and long lasting [7].

There is now also emerging evidence that prostatic injections of BoNTA have a marked effect of improving urinary flow and lower urinary tract symptoms (LUTS) in men who are poor candidates for prostate surgery [8–10]. It is far too early to know if this will ever become a licensed treatment for benign prostatic hyperplasia, but some interesting experimental studies in dogs and most recently rats have shown that the observed atrophy that affects the gland is largely the result of apoptosis, a process that appears to be related to sympathetic chemodenervation [11].

So far, the changes in human bladder following BoNTA injections that can be associated with a successful outcome are a reduction in TRPV1 and P2X expression [12] in the suburothelial innervation. However, from the sparse data available, no definitive conclusions can be drawn about degeneration or reinnervation phenomena in the human bladder wall following BoNTA injections as of yet. The only data existing are those by Haferkamp et al [13], who showed nearly no structural differences of the detrusor before and after BoNTA injections. Contrary to studies on striated muscle, no increase in axonal sprouting after BoNTA injections was observed, indicating pathophysiologically different reactions to the toxin either between striated muscle and smooth muscle or between different treated diseases [13]. Based on laboratory experiments, it seems highly probable that the toxin blocks release of various N-ethylmaleimide-sensitive factor attachment protein receptor–dependent neurotransmitters at least in the short term, but an explanation for the effects of BoNTA, which have been observed to last between 9 and 10 mo, is still awaited. In keeping with the evidence of extensive apoptosis that underlies the mechanism of action in the prostate, a recent rat study could demonstrate (repeated) injections 10 U of BoNTA into the bladder wall to induce marked apoptotic cell losses in the bladder urothelium and suburothelium but not the detrusor [14]. We set out to address the question of whether a comparable apoptotic cell loss could be observed in the human urothelial and suburothelial layer after intradetrusor BoNTA injections.

2. Patients and methods

2.1. Patients and injection technique

In this prospective study, bladder biopsies were taken from 12 overactive bladder (OAB) patients with neurogenic detrusor overactivity resulting from MS (eight women, four men; median age: 45 yr [interquartile range (IQR): 42–50]) and seven controls (all women; median age: 56 yr [IQR: 43–64]). The 12 patients were part of the group of 43 whose excellent clinical response to detrusor injections of BoNTA has already been reported [3]. Briefly, detrusor overactivity was proven urodynamically in all, and treatment with more than one antimuscarinic drug for at least 3 mo failed. Patients were treated according to a research protocol approved by the local research ethics committee.

Intradetrusor BoNTA injections were performed in an outpatient setting using the previously described minimally invasive technique [15], injecting 300 U of BoNTA (BOTOX; Allergan, Irvine, CA, USA) at 30 different sites in the detrusor, sparing the trigone [1]. Flexible cystoscopic bladder biopsies were obtained at baseline pretreatment and during check flexible cystoscopy at 4 and 16 wk after each treatment session. Biopsies were obtained from a consistent bladder area 2 cm above and lateral to the ureteric orifices. Control tissue was obtained endoscopically from seven patients (all women; median age: 56 yr) being examined under anaesthesia prior to pelvic floor repair procedures. They had macroscopically normal bladders, no symptoms of OAB, and sterile urine at the time of endoscopy.

2.2. Outcome measures

Outcome measures were the number of apoptotic cells in the bladder of OAB patients before and 4 wk after first intradetrusor BoNTA injections and apoptotic cells in the bladder of controls.

2.3. Immunoreaction

Biopsy specimens were snap frozen in liquid nitrogen, embedded in optimal cutting temperature medium, and stored at −60 °C. Three sections per specimen were cut in a cryostat at 10 μm thickness and collected on 3-aminopropyltriethoxysilane-coated superfrost slides.

Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) staining, a well-established method for the rapid identification of apoptotic cells, was performed using the In Situ Cell Death Detection Kit, Fluorescein (FITC; Roche Diagnostics, Burgess Hill, UK). Frozen sections were fixed, permeabilised, and labelled following the manufacturer's instructions using a 1:2 TUNEL dilution. Nuclei were counterstained with 4′, 6-diamidino-2-phenylindole (DAPI; Invitrogen, D1306, 1:50 000) during incubation in a humidified atmosphere in the dark. One negative control (a section without terminal deoxynucleotidyl transferase) was included in each experimental set. Human glioblastoma multiforme tissue (malignant glial brain tumour) was used as a positive control. Slides were cover slipped using Citifluor mounting medium (Agar Scientific, Stansted, UK). Immunolabelled sections were examined using a laser scanning confocal microscope (Zeiss LSM-510 META, Germany) equipped with an argon laser (458 nm, 488 nm, 514 nm), a helium-neon laser (543 nm, 633 nm), and a 405-nm diode laser. Fluorescence was excited at 488 nm (FITC) and 405 nm (DAPI) and 543 nm (Cy3) and recorded with separate detectors.

Only those sections that displayed a complete and strictly transversal cross-section of the bladder urothelium and suburothelium were chosen for numeric determination of apoptotic cells. Whole-section images were taken with a ×20 objective blinded to the sample. We first calibrated the detection system on a reference section and reused the