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Effect of botulinum-A toxin to cremaster muscle: an experimental study

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Abstract Objective: A controversy exists on the definition, etiology and treatment of the retractile testes. In the present experimental study, we aimed to show the effect of botulinum-A toxin (Botox) on cremasteric muscle of a rat, and whether it may be an alternative to surgical treatment of retractile testis. Methods: Ten Wistar rats were used in the study. By stimulating cremasteric reflex, five compound muscle action potentials (CMAP) of the right and left cremasteric muscles of each rat were recorded using surface electrodes. Intramuscular injection of botulinum-A toxin was done to the right side. Saline was injected to the left cremasteric muscles, and the left side also served as control. CMAP of the cremasteric muscles were recorded 45 days after the injection. Statistical analysis was done using Wilcoxon Signed rank test. Results: Mean CMAP of the right side was 3.25 ± 1.39 μV before the injection and 0.44 ± 0.25 μV after botulinum-A toxin injection. The difference was statistically significant ($p < 0.05$). Mean CMAP on the left side was 3.48 ± 0.32 μV and 3.14 ± 1.12 μV at baseline and the end of the study, respectively. The difference was not statistically significant ($p > 0.05$). Conclusion: The botulinum-A toxin paralyzes the cremasteric muscles of the rats. As cremasteric hypertonicity is accepted as one of the reasons for retractile testes, botulinum-A toxin injection to cremasteric muscles may be helpful in diagnosis and may be an alternative to surgical treatment of this pathology in repeated dosages. Long-term evaluation of this paralysis is necessary.

Keywords Retractile testis · Botulinum toxin · Experimental study · EMG · Muscle action potential

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Introduction

A controversy exists on the definition, etiology and treatment of the retractile testes among different investigators. Some authors accept it as resulting from a hyperactive reflex while others do not [4, 20]. Human chorionic gonadotropin (hCG) injection and/or orchidopexy are the treatment modalities of retractile testes, although some authors propose to wait until puberty [2, 8, 15]. Whether hyperactive or not, it is widely accepted that m. cremaster is involved in the pathology. Botulinum toxin type A (Botox-A) is a muscle paralytic agent which is used in spasmodic dysphonia, strabismus, blepharospasm, oromandibular dystonia, craniocervical dystonia, torticollis, hemifacial spasm, focal hand dystonia, and anismus [18].

In this experimental study, we aimed to show the effect of Botulinum toxin injection to cremasteric muscle and whether it is an alternative for treatment of retractile testes by paralyzing the cremaster muscle and allowing the testes to remain in the scrotum of the rats.

Materials and methods

A total of ten adult male Wistar rats weighing 250 ± 15 g were the subjects of this study. The animals were kept under standardized conditions, were fed standard rat chow, and were allowed water ad libitum. The study was approved by the Medical Faculty Ethical Committee. The rats were anesthetized with an intramuscular injection of 40 mg/kg ketamine hydrochloride (Ketalar, Eczacibasi, Turkey) and bilateral inguinal transversal incisions were made after shaving and cleaning the skin with povidone iodine. The electrical activity of the cremasteric muscles was recorded in ten rats. The localization of cremasteric muscle and reflex activity was confirmed by observing the upward contraction of the testis as a response to

the bipolar stimulation of the dorsalis penis nervi or the thigh. Electric stimuli lasting 0.2 ms of constant square wave pulses were applied to either the medial aspect of the thigh or dorsal surface of the penis (genitofemoral nerve) transcutaneously with bipolar needle electrodes (concentric needle electrode, stainless steel). The stimulus threshold was 30 mA for the thigh and dorsalis penis. In order to prevent habituation, different stimuli amplitudes and irregular intervals, with interstimulus lasting at least 3 s, were applied to elicit the cremasteric reflex. Compound muscle action potentials (CMAP) of the cremasteric muscles were recorded with surface electrodes (disposable, 10-mm-diameter Ag/AgCl electrodes). The researchers observed the contraction of cremasteric muscle in every successive evoked stimulus in addition to the CMAP recording. A ground electrode was placed near the surface electrodes. The impedance of recording electrodes was below 5 ohms. Five successive CMAPs were recorded for each recording (50 Hz–5 kHz band pass filter, 50–200 μV per division amplifier display gain, 200–1000 ms sweep time). The CMAPs were averaged and evaluated for their amplitudes. The measurement was done on Keypoint 4c (Dantec, Denmark). Botulinum toxin (Botox, Allergan, Ireland) was applied as 0.5 ml (10 U/ml) to the right side obliquus internus and cremasteric muscles by a 26-gauge needle. The cremaster muscle of the rat consists of two layers nearly 0.2 mm thick. Outer circular fibers derive from the internal abdominal oblique muscle and inner longitudinal muscles from the transversus abdominus muscle. We injected botulinum toxin to the all layers of the right inguinal ring deeply enough to reach all fibers [9]. Saline was injected with a 26-gauge needle in left-side muscles. The skin was closed with 3-0 silk sutures. The CMAP potentials were recorded 45 days after application of botulinum toxin as previously described. The left side served as the control group. The difference in CMAP was examined as the consequent effect of botulinum toxin. Statistical analysis was done by Wilcoxon Signed Rank test and $p < 0.05$ was accepted as statistically significant.

Results

The CMAP of the cremasteric muscles was recorded before and 45 days after the application of the botulinum toxin. The mean CMAP of the right side was 3.25 ± 1.39 μV before the injection and 0.44 ± 0.25 μV after botulinum toxin injection. The difference was statistically significant ($p < 0.05$). The mean CMAP of the left side was $3.48 \pm 0{,}32$ μV before the injection and 3.14 ± 1.12 μV after the injection of saline. The difference was not statistically significant ($p > 0.05$).

The failure of contraction in cremasteric muscle and reflex activity was maintained in right-side testes after botulinum toxin injection. Inhibition of testicular movements was observed after bipolar stimulation of the dorsalis penis nervi or the thigh.

Discussion

We obtained inhibition of cremasteric reflex by botulinum toxin injection to the cremasteric muscles of the rats. Forty-five days after the injection, there was a significant decline in CMAP of the cremasteric muscles. This was statistically significant ($p < 0.05$).

Testicular retraction results from the cremaster muscle activity either spontaneously or provoked, and this seems to be a protective reflex [15].Treatment is controversial and some authors recommend waiting until puberty in the case of retractile testis, while the others reported that tubular degeneration such as cryptorchid testes suggests the need for either hormonal or surgical therapy in retractile testes [4, 8]. Both hormonal and surgical treatments have various early and late complications. An inflammation-like reaction and a reduced adult size of the testes is reported after hCG treatment in undescended testicles [19]. Similarly, apoptotic loss of spermatogonia after hCG treatment of undescended testis has been reported [7]. Surgical therapy is performed under general anesthesia using a transversal inguinal approach and complications of orchidopexy include retraction of the testis, postoperative torsion, hematoma formation, ilioinguinal nerve injury damage to the vas deferens, occlusion of vas deferens, atrophy of the testis and vascular injuries [10].

Since the use of botulinum toxin for strabismus, its use in several conditions resulting from involuntary muscle spasms has been reported [5, 18]. Botulinum neurotoxin is produced by Gram-negative anaerobic bacterium, *Clostridium botulinum*. The neurotoxin is synthesized in seven different serotypes: A, B, C, D, E, F and G; serotype A is the most potent [6].

Botulinum neurotoxin acts selectively on peripheral cholinergic nerve endings to inhibit acetylcholine release. It is most potent at the neuromuscular junction, but also has the power of inhibiting transmitter release from pre- and postganglionic cholinergic nerve endings of the autonomic nervous system [6].

Botulinum A toxin is used in neurological fields. Schurch et al. successfully used Botulinum toxin in detrusor-sphincter dyssynergia in spinal cord pathologies [17]. Similarly, Schulte et al. used Botulinum toxin in 17 children with detrusor hyperreflexia due to myelomeningocele and observed improvement in bladder capacity, pressure and compliance [16].

Botox-A may be an alternative to surgical therapy of retractile testis. It can be applied under local anesthesia. It is well tolerated and has no gross side effects.

The limitation of this treatment may be its short activity period. The muscle fiber atrophy is reversible in a period of 4–6 months [3]. This can be achieved by repeated injections. Messineo et al. reported a 2- 14-month duration of efficiency in internal anal sphincter achalasia treated with Botox-A after a second injection [14].

The cremaster muscle plays a role in the thermoregulation of the testis. Kojima et al. showed a decrease in testicular weight and degenerative changes of the seminiferous tubules after the impairment of the cremaster reflex [11]. In surgical corrections of testicular pathologies, dissection or division of the muscle is permanent, but the temporary effect of Botox-A may be an advantage, as its effect lasts in 4–6 months. We cannot discuss the long-term effect of Botox-A and contractility of the cremaster muscle as 45 days is insufficient to determine the long-term effects.

According to Koloğlu et al., retractile testis is not a result of the activation of cremasteric reflex, and instead it is believed that retractile testis may be a result of alterations within the contractile properties of cremaster muscle [12]. This also may be achieved by the use of Botox-A, as it paralyzes the cremaster muscle.

If used in clinical cases, Botox-A may also be helpful in identifying the etiology of retractile testis. Atwell has suggested that persistence of the processus vaginalis may be a reason for retractile testis [1]. Observing the localization of the testicles after cremaster muscle paralysis may help to clarify the cause.

This experimental pilot study shows that local injection of Botox-A in m. obliquus internus and m. cremaster may be effective on retractility of the testis, although it is temporary. Clinical application and longer follow-up periods for the observation of the effect of Botox-A on muscles is necessary to define the exact time and whether or not multiple injections are required.

References

1. Atwell JD (1985) Ascent of the testis: fact or fiction. Br J Urology 57: 474
2. Belman AB (1988) Acquired undescended testis: effects of human chorionic gonadotropin. J Urol 140: 1189
3. Borodic GE, Ferrante RJ, Pearce LB, Alderson K (1994) Pharmacology and histology of the therapeutic application of botulinum toxin. In: Jankovic J, Hallett M (ed) Therapy with botulinum toxin. Marcel Dekker, New York, p 119
4. Caucci M, Barbatelli G, Cinti S (1997) The retractile testis can be a cause of adult infertility. Fertil Steril 68: 1051
5. Childers MK, Wilson DJ, Simison D (1999) Introduction. In: Childers MK, Wilson DJ, Simison D (eds) Use of botulinum toxin type A in pain management. Academic Information Systems, Columbia, p 1
6. Coffield JA, Considine RB, Simpson LL (1994) The site and mechanism of action of botulinum neurotoxin. In: Jankovic J, Hallett M (eds) Therapy with botulinum toxin. Marcel Dekker, New York, p 3
7. Dunkel L, Taskinen S, Hovatta O, Tilly JL, Wikstr S (1997) Germ cell apoptosis after treatment of cryptorchidism with human chorionic gonadotropin is associated with impaired reproductive function in the adult. J Clin Invest 100: 2341
8. Han SW, Lee T (1999) Pathological difference between retractile and cryptorchid testes. J Urol 162: 878
9. Hebel R, Stromberg MW (1986) Anatomy and embryology of the laboratory rat. Section F: male reproductive organs. BioMed Verlag, Wörthsee, p 70
10. Hutson JM (1995) Orchidopexy. In: Spitz L, Coran AG (eds) Operative surgery. Chapman and Hall, London, p 717
11. Kojima M, Ohe H (1986) Experimental study on the regulation of testicular function by the cremaster reflex in rats. Tohoku J Exp Med 150: 175
12. Koloğlu M, Tanyel C, Anlar B, Büyükpamukçu N (2001) Cremasteric reflex and retraction of a testis. J Pediatr Surg 36: 863
13. Lange DJ (1994) Systemic effects of botulinum toxin. In: Jankovic J, Hallett M (eds) Therapy with botulinum toxin. Marcel Dekker, New York, p 109
14. Messineo A, Codrich D, Monai M, Martellossi S, Ventura A (2001) The treatment of internal anal sphincter achalasia with botulinum toxin. Pediatr Surg Int 17: 521
15. Rajfer J (1998) Congenital anomalies of the testis and scrotum. In: Walsh PC, Retik AB, Vaughan ED, Wein AJ (eds) Campbell's Urology. W.B. Saunders, Philadelphia, p 2172
16. Schulte H, Michael T, Schobert J, Stolze T, Knispel HH (2002) Efficacy of botulinum-A toxin in children with detrusor hyperreflexia due to myelomeningocele: preliminary results. Urology 59: 325
17. Schurch B, Stohrer M, Kramer G, Schmid DM, Gaul G, Hauri D (2000) Botulinum A toxin for treating detrusor hyperreflexia in spinal cord injured patients: a new alternative to anticholinergic drugs? Preliminary results. J Urol 164: 692
18. Shaari CM, Sanders I (1993) Quantifying how location and dose of botulinum toxin injections affect muscle paralysis. Muscle Nerve 16: 964
19. Toppari J, Kaleva M (1999) Maldescendus testis. Horm Res 51: 261
20. Way GD, Hutson JM (1992) Is the retractile testis a normal physiological variant or an anomaly that requires active treatment? Pediatr Surg Int 7: 249