Original Article

Evaluation of Pain and Prostate Volume of Dogs Treated with Intra-prostatic Injection of Botulinum Toxin Type A

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Abstract

Recently, alternative medical treatment of benign prostatic hyperplasia (BPH) using intra-prostatic injection of botulinum toxin type A (BT-A) has been reported in men and mice. However, in terms of animal welfare, it remains questionable when applied to clinical use. This study aimed at evaluating the effects of intra-prostatic injection of BT-A (Botox, Allergan, USA) in dogs. Six healthy intact males with no clinical signs of BPH received 100 units of BT-A by ultrasound-guided transabdominal intra-prostatic injection (50 units of each prostatic lobe). Pain scoring, evaluation of white blood cell counts, cortisol concentrations, and observation of urination behaviour were recorded before (24 and 12 h) and at 2, 4, 6, 12, 24, 48 and 72 h after injection. Effects of BT-A on prostate volume reduction were assessed by ultrasound scan 24 h before and on days 30, 60 and 90 after injection. This study was reviewed and approved by the Animal Committee of the university. No significant differences in pain score, inflammation condition, cortisol level and urination behaviour were observed. In addition, no significant changes in prostate volume were recorded at any time points observed after BT-A injection ($p > 0.05$). These findings indicated that, during 72-h after treatment, BT-A caused no acute discomfort/pain and stress to the animals. Moreover, the injection of BT-A at dosage used in this study did not affect prostatic volume in the dogs.

Keywords: botulinum toxin, dog, prostate

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Introduction

Benign prostatic hyperplasia (BPH) commonly occurs in middle-aged to older intact male dogs (Johnston et al., 2001). Most intact male dogs that reach 5 years of age have more than 80% evidence of developing BPH (Wiebe and Howard, 2009). Benign prostatic hyperplasia in dog is suggested to result from hormone alteration and/or inflammation (Briganti et al., 2009). The progression and development of BPH consist of increased cell proliferation (Ventura et al., 2002) together with decreased cell death and apoptotic activity (Isaacs, 1983). These processes cause enlargement of the gland, thus increasing proximal urethra pressure, decreased urinary flow rate and obstruction of urinary flow (Kaszkiel et al., 1997; Ventura et al., 2002). Alternative medical treatments of canine BPH include estrogenic agents, progestins (Ventura et al., 2002), GnRH agonists (Johnston et al., 2001), finasteride (Kamolpatana et al., 1998; Smith, 2008) and bilateral orchidectomy (Roylance et al., 1995). To date, estrogenic agents and progestins are not recommended due to their side effects of long-term administration (Ventura et al., 2002). Continuous use of finasteride, a 5 alpha-reductase inhibitor, is effective for treatment of human and canine BPH. The 5 alpha-reductase is an enzyme that converts testosterone to dihydrotestosterone, a main substance that causes development of BPH (Isaacs, 1983; McVary, 2007). Because finasteride has no effects on testosterone levels, libido, and semen quality in dog (Wiebe and Howard, 2009), it is therefore suggested to treat stud dogs suffering from BPH. Nevertheless, daily drug administration of finasteride may not be practical for some circumstances and discontinuation of finasteride results in recurrence of prostate enlargement. For many years, surgical castration is the traditional choice for BPH dogs, although it is not recommended for stud dogs with high breeding value, and it is a matter of fact that some dog owners refuse surgical intervention for certain reasons. The use of GnRH agonist, i.e. deslorelin, has been registered for veterinary use and is becoming more popular in small animal reproduction practice including BPH because of its long-term efficacy after a single subcutaneous implantation (Ponglowhapan et al., 2002; Ponglowhapan and Lohachit, 2010). However, deslorelin adversely affects semen quality and currently not available worldwide in veterinary market (Wiebe and Howard, 2009). Therefore, other alternative treatments for canine BPH remain a subject to be further investigated.

In humans, botulinum toxin type A (BT-A) has been clinically used in urogenital disorders (Chuang et al., 2006; Oeconomou et al., 2008; Nishiyama et al., 2009). Intra-prostatic injection with BT-A has been studied in humans (Chuang et al., 2005) and rats (Nishiyama et al., 2009). Studies have shown that the intra-prostatic injection of BT-A reduces prostatic volume and induces glandular apoptosis (Chuang et al., 2008; Oeconomou et al., 2008). In dogs, the injection of BT-A into prostates reduces contractile function while maintaining relaxation response of the prostate and these effects make botulinum toxin a viable option in managing prostate-related symptoms (Lin et al., 2007). Although it has been reported in humans that BT-A inhibits COX-2 release, a mediator of pain and inflammation, from the prostate (Chuang et al., 2008; Silva et al., 2009), it remains to be clarify in terms of animal welfare, particularly when applied to clinical practice if intra-prostatic injection with chemicals, i.e botulinum toxin, will cause pain and discomfort to animals. A further study on pain, stress, observation of urination behaviour and prostate volume following intra-prostatic injection of BT-A in dogs is required to determine the potential use of this approach in BPH dogs.

Materials and Methods

***Study design*:** Dogs were given intra-prostatic injection of botulinum toxin type A. Pain scoring, stress assessment as evaluated by white blood cell counts and cortisol concentrations, and observation of urination behaviour were recorded before (24 and 12 h) and at 2, 4, 6, 12, 24, 48 and 72 h after injection. Effects of BT-A on prostate volume reduction were assessed by ultrasound scan 24 h before and on days 30, 60 and 90 after injection.

***Animals*:** This study was reviewed and approved by the Animal Committee of the Faculty of Veterinary Science, Chulalongkorn University (Reg. No. 11310084). Six healthy intact male beagles, aged between 2 and 6 years and weighing between 10 and 15 kg, with no clinical signs or any abnormality of the urogenital tract were included in the study. The dogs were kept in open house, fed on commercial diet and given water ad libitum. They stayed at room temperature and normal humidity. All dogs were familiar with their environment and the animal caretakers.

***Intra-prostatic injection*:** All dogs were given general anesthesia (propofol, 6 mg/kg IV) 15 min before intra-prostatic injection. Aseptic techniques were performed at the injection site as a standard practice. The dog lied on dorsal recumbency position. With the aid of transabdominal ultrasonographic guidance, 20-gauge needle, 9-cm long (Terumo® spinal needle) was introduced into the center of each of the prostatic lobes (right versus left). During the insertion of needle into each prostate lobe the ultrasound located the position of prostate gland until the needle tip located at the center of the gland parenchyma. A vial of BT-A (Botox®) (100 units) was dissolved in 3 mL of normal saline. Intra-prostatic injection of Botox® was done slowly. Each lobe of prostate gland received 50 units of Botox® in a total volume of 1.5 mL solution.

***Assessment of inflammatory condition*:** Profiles of complete blood count were assessed at 12 and 24 h before intra-prostatic injection and on 48 and 72 h after treatment. Number of white blood cells and percentage of differentiated white blood count were evaluated.

***Assessment of stress condition*:** Stress condition was determined by serum cortisol concentrations (Devitt et al., 2005). Serum cortisol concentrations were measured before intra-prostatic injection (12 and 24 h)

as a baseline level and at 2, 4, 6, 12, 24 and 48 h after treatment. Serum was harvested and stored at -80°C for evaluation of cortisol levels by radioimmunoassay.

***Pain score observation*:** All dogs were observed for pain and discomfort before and after intra-prostatic injection of Botox® using a modified pain scale (Table 1) derived from previous studies (Devitt et al., 2005; Wagner et al., 2008; Case et al., 2011). Pain assessment was scored and recorded at 2, 4, 6, 12, 24, 48 and 72 h (Case et al., 2011) after treatment and before blood collection on each occasion. Total pain score ranged between 0 (least painful) and 19 (most painful). Pain scores of each dog was summarized and a total pain score more than 8 out of 19 is considered painful and therefore the dog will be given analgesic (tramadol 4 mg/kg S/C).

Table 1 Pain scoring system

Variable	Score	Criteria
Overall	0	Happy or bouncy
	1	Quiet
	2	Non-responsive to surroundings
	3	anxious or fearful
	4	Depressed or non-responsive to stimulation
Vocalization	0	Not vocalization
	1	During palpation of injection site
	2	Intermittent
	3	Continuous
Movement	0	Normal
	1	Stiff, ataxia or position change
	2	Show or reluctance to rise or site, lame
Vomiting	0	No
	1	Yes
Palpation of the injection site	0	No response
	1	Turn head
	2	Evade
	3	Guarding wound, tries to bite
Heart rate (compare with baseline rate)	0	<20% increase
	1	>20% to ≤ 50% increase
	2	>50% to ≤ 100% increase
	3	>100% increase
Respiratory rate(compare with baseline rate)	0	<20% increase
	1	>20% to ≤ 50% increase
	2	>50% to ≤ 100% increase
	3	>100% increase

The table is modified from studies by Devitt et al. (2005), Wagner et al. (2008) and Case et al. (2011).

***Evaluation of the prostate volume by ultrasonography*:** A transabdominal ultrasound probe was placed parallel to the prepuce and the bladder to locate and visualize the prostate longitudinally and transversely. Prostatic size (length, width and depth), shape, parenchymal echogenicity and volume were assessed before (24 h) and after treatment (days 30, 60 and 90). Canine prostatic volume was estimated using the previously reported formula by Kamolpatana et al. (2000). Prostatic volume (cm^3) = [(L × W × D) / 2.6] + 1.8.

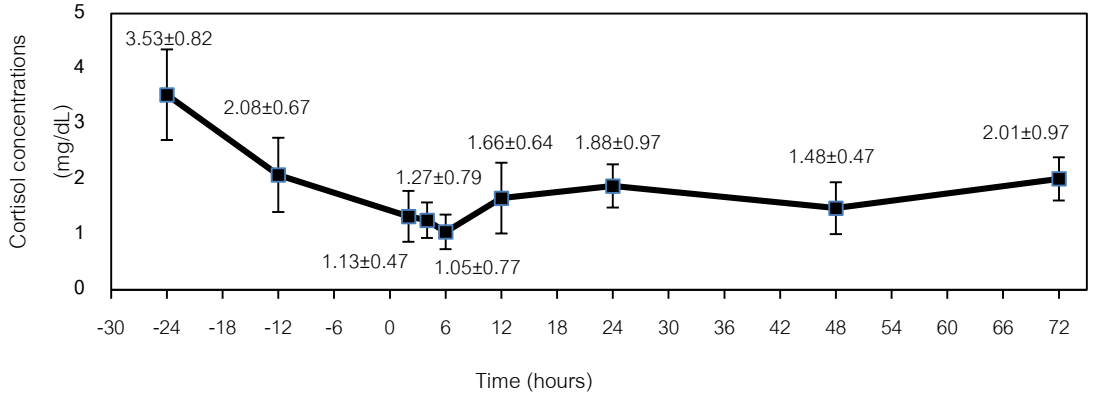

Figure 1 Serum cortisol concentrations of dogs before and after treatment (0 = injection time)

***Statistical analysis*:** Statistical analyses of inflammatory condition, cortisol concentration, pain score and prostatic volume were done by Kruskal-Wallis Test for comparison between before- and after-treatment values. Values of $p \leq 0.05$ were considered statistically significant.

Results

***Inflammatory condition*:** The profiles of complete blood count, blood biochemistry, number of white blood cells and percentage of differentiated white blood cells before and after intra-prostatic injection were within normal ranges and were not different by treatment. The total white blood cell count (p = 0.08) and the percentage of neutrophil (p = 0.21) did not differ between before and after intra-prostatic injection.

***Stress assessment*:** The cortisol levels before and after treatments are shown in Figure 1. In this study, the levels ranged between 0.28 and 4.4 mg/dL. There were no significant differences in the levels between any time points (p = 0.15).

***Pain assessment*:** Overall, the animals showed no signs of pain or discomfort as indicated in Table 1. The scores during the entire study were zero. No changes in urination behavior were observed between pre- and post-injection of BT-A into the prostates.

***Prostatic volume*:** The volume of prostates was evaluated by transabdominal ultrasound scan. No significant differences in the prostate volume were observed before (ranged between 9.6 and 31.96 cm^3) and after treatment (ranged between 9.3 and 37.8 cm^3) ($p > 0.05$).

Discussion

Botulinum toxin type A has been successfully applied to treat many urogenital symptoms in humans (Yokoyama et al., 2002) including patients with symptomatic BPH (Chuang et al., 2005). It is logical to extend this approach to dogs as BPH is a common aging disease spontaneously observed in both humans and dogs.

In this study, the injection procedure of BT-A into the canine prostates was possible and repeatable with the aid of transabdominal ultrasound guidance. BT-A was injected into each prostate lobe to ensure distribution of the chemical as anatomically the dog prostate has 2 separate lobes. No differences in pain score, stress assessment (evaluated by white blood cell counts and serum cortisol concentrations), and urination behaviors were observed. These indicated that the procedures used in the study and the intra-prostatic injection of BT-A were acceptable in clinical practice in terms of animal welfare. However, clinical investigation into the intra-prostatic injection with BT-A in BPH dogs remains to be evaluated as this study used clinically healthy dogs and the results showed no reduction in prostate volume during the 90-day observation period post-injection. Similarly, a recent study in dogs demonstrated that changes in the volume of prostate were not significant after the intra-prostatic injection of another type of BT-A (Dysport®) (250 units per animal) (Mostachio et al., 2012). Conversely, a reduction in prostatic volume following the intra-prostatic injection of BT-A has been significant in men and mice (>50%) (Maria et al., 2003; Chuang et al., 2006). Different results in BT-A treatment of BPH between humans and dogs are of interest for further investigation although dog has long been served as an animal model of human prostate research. In conclusion, the administration of 100 units of BT-A (Botox®) injected into the dog prostates resulted in no significant reduction in the gland and no pain/discomfort of the animals was clinically observed.

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References

Briganti A, Capitanio U, Suardi N, Gallina A, Salonia A, Bianchi M, Tutulo M, Girolama VD, Guazzoni G, Rigatti P and Montorsi F 2009. Benign prostatic hyperplasia and its aetiologies. Eur Urol Suppl. 8:865-871.

Case JB, Marvel SJ, Boscan P and Monnet EL 2011. Surgical time and severity of postoperative pain in dogs undergoing laparoscopic ovariectomy with one, two, or three instrument cannulas. JAVMA. 239:203-208.

Chuang YC, Chiang PH, Huang CC, Yoshimura N and Chancellor MB 2005. Botulinum toxin type A improves benign prostatic hyperplasia symptoms in patients with small prostates. Urology. 66:775-779.

Chuang YC, Tu CH, Huang CC, Lin HJ, Chiang PH, Yoshimura N and Chancellor MB 2006. Intraprostatic injection of botulinum toxin type A relieves bladder outlet obstruction in human and induces prostate apoptosis in dogs. BMC Urology. 6:1-6.

Chuang YC, Yoshimura N, Huang CC, Wu M, Chiang PH and Chancellor MB 2008. Intraprostatic botulinum toxin A injection inhibits cyclooxygenase-2 expression and suppresses prostatic pain on capsaicin induce prostatitis model in rat. J Urol. 180:742-748.

Devitt CM, Cox RE and Hailey JJ 2005. Duration, complications, stress, and pain of open ovariohysterectomy versus a simple method of laparoscopic-assisted ovariohysterectomy in dogs. JAVMA. 227:921-927.

Isaacs JT 1983. Changes in dihydrotestosterone metabolism and the development of benign prostatic hyperplasia in the aging beagle. J Steroid Biochem. 18:749-757.

Johnston SD, Kustritz MVR and Olson PNS 2001. Sexual differentiation and normal anatomy of the dog. In: Canine and Feline Theriogenology. USA: Elsevier. P257-286.

Kamolpatana K, Johnston SD, Hardy SK and Castner S 1998. Effect of finasteride on serum concentrations of dihydrotestosterone and testosterone in three clinically normal sexually intact adult male dogs. AJVR. 59:762-4.

Kamolpatana K, Johnston GR and Johnston SD 2000. Determination of canine prostatic volume using

transabdominal ultrasonography. Vet Radiol Ultrasound. 41:73-7.

Kaszkiel ATN, Iulio JLD, Li CG and Rand MJ 1997. Characterization of excitatory and inhibitory transmitter systems in prostate glands of rats, guinea pigs, rabbits and pigs. EJP. 337:251-258.

Lin ATL, Yang AH and Chen KK 2007. Effect of botulinum toxin A on the contractile function of dog prostate. Eur Urol. 52:582-589.

Maria G, Brisind G, Civello IM, bentivoglio AR, Sganga G, Albanese A 2003. Relief of botulinum toxin of voiding dysfunction due to benign prostatic hyperplasia: results of a randomized, placebo-controlled study. Urology. 62:259-264.

McVary KT 2007. A Review of combination therapy in patients with benign prostatic hyperplasia. Clin Ther. 29:387-398.

Mostachio GQ, Apparicio M, Motheo TF, Alves AE, Vicente WRR. 2012. Intra-prostatic injection of botulinum toxin type A in treatment of dogs with spontaneous benign prostatic hyperplasia. Anim Reprod Sci. 133:224-228.

Nishiyama Y, Yokoyama T, Tomizawa K, Okamura K, Yamamoto Y, Matsui H, Oguma K, Nagai A and Kumon H 2009. Effects of purified newly developed botulinum neurotoxin type A in rat prostate. Urology. 74:436-439.

Oeconomou A, Madersbacher H, Kiss G, Berger TJ, Melekos M and Rehder P 2008. 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. 54:765-777.

Ponglowhapan S, Lohachit C, Swangchan-uthai T and Trigg TE 2002. The effects of GnRH agonist on prostatic volume in dogs. Proceeding of the 3rd Annual Symposium of the European Veterinary Society for Small Animal Reproduction (EVSSAR). Liege,Belgium. May 10-12:150.

Ponglowhapan S and Lohachit C 2010. Clinical use of GnRH agonist Deslorelin in benign prostatic hyperplasia in dogs. Proceedings of the 7th Annual Symposium of the European Veterinary Society for Small Animal Reproduction (EVSSAR). Louvain-La-Neuve, Belgium. May 14-15: 93.

Roylance P, Gibelin B and Espie J 1995. Current treatment of BPH. Biomed & Pharmacother. 49:332-338.

Silva J, Pinto R, Carvallho T, Coelho A, Avelino A, Dinis P and Cruz F 2009. Mechanisms of prostate atrophy after glandular botulinum neurotoxin type A injection: An experimental study in the rat. Eur Urol. 56:134-141.

Smith J 2008. Canine prostatic disease: A view of anatomy, pathology, diagnosis, and treatment. Theriogenology. 70:375-383.

Ventura S, Pannefather JN and Mitchelson F 2002. Cholinergic innervation and function in the prostate gland. Pharmacol & Ther. 94:93-112.

Wanger AE, Worland GA, Glawe JC and Hellyer PW 2008. Multicenter, randomized controlled trial of pain-related behaviors following routine neutering in dogs. JAVAM. 233:109-115.

Wiebe VJ and Howard JP 2009. Pharmacologic advances in canine and feline reproduction. TCAM. 24:71-99.

Yokoyama T, Kumon H, Smith CP, Somogyi GT and Chancellor MB 2002. Botulinum toxin treatment of urethral and bladder dysfunction. Acta Med Okayama. 56:271-277.

บทคัดย่อ

การศึกษาผลในด้านความเจ็บปวดและปริมาตรต่อมลูกหมาก ภายหลังการฉีดสารโบทูลินัมท็อกซิน ชนิดเอ เข้าสู่ต่อมลูกหมากสุนัข

ปิณฑิรา เที่ยงเธียรธรรม เกวลี ฉัตรดรงค์ ศุภวิวัฒน์ พงษ์เลาหพันธุ์*

มีรายงานการศึกษาถึงความเป็นไปได้ในการฉีดสารโบทูลินัมท็อกซินเข้าสู่ต่อมลูกหมากเพื่อลดขนาดต่อมลูกหมากในคนและหนู การศึกษานี้มีวัตถุประสงค์เพื่อประเมินผลของการฉีดสารโบทูลินัมท็อกซิน ชนิดเอ (BT-A) เข้าสู่ต่อมลูกหมากสุนัข โดยทำการประเมินในด้านความเครียด ความเจ็บปวด และปริมาตรต่อมลูกหมากภายหลังการฉีด ทำการศึกษาในสุนัขบีเกิ้ลสุขภาพดีที่ยังไม่ได้ทำหมันจำนวน 6 ตัว ฉีด BT-A จำนวน 100 ยูนิตเข้าต่อมลูกหมากผ่านทางผนังช่องท้องโดยใช้เครื่องอัลตราซาวน์ช่วยในการมองภาพขณะฉีด ทำการจดบันทึกคะแนนความเจ็บปวดจากพฤติกรรมและท่าทางขณะปัสสาวะ จำนวนเม็ดเลือดขาวในกระแสเลือด และระดับคอร์ติซอล ก่อน (24 และ 12 ชั่วโมง) และหลัง (2 4 6 12 24 48 และ 72 ชั่วโมง) การฉีด BT-A และคำนวณปริมาตรต่อมลูกหมากจากภาพอัลตราซาวน์ก่อน (24 ชั่วโมง) และหลัง (30 60 90 วัน) การฉีด การศึกษานี้ได้ผ่านการพิจารณาจรรยาบรรณการใช้สัตว์ทดลองจากสถาบันที่ทำการศึกษา จากการศึกษาพบว่าค่าคะแนนความเจ็บปวดจากพฤติกรรมและท่าทางขณะปัสสาวะ จำนวนเม็ดเลือดขาวในกระแสเลือด และระดับคอร์ติซอลไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติระหว่างก่อนและหลังฉีด**ใน**ทุกช่วงเวลาที่ทำการจดบันทึกตลอดระยะเวลา 72 ชั่วโมง นอกจากนี้ยังไม่พบการเปลี่ยนแปลงของปริมาตรต่อมลูกหมากระหว่างก่อนและหลังฉีด จากการศึกษาสรุปได้ว่าการฉีด BT-A ด้วยวิธีดังกล่าวไม่ก่อให้เกิดอาการเจ็บปวดหรือความเครียดต่อสุนัข และไม่มีการเปลี่ยนแปลงของขนาดต่อมลูกหมากในช่วงระยะเวลาที่ศึกษา

คำสำคัญ: โบทูลินัมท็อกซิน สุนัข ต่อมลูกหมาก

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