Evaluation of Pain and Prostate Volume of Dogs Treated with

Intra-prostatic Injection of Botulinum Toxin Type A

Pintira Thiangthientham  Kaywalee Chatdarong  Suppawiwat Ponglowhapan*

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 (Isacs, 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 (Isacs, 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 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 (Ponglowhanap et al., 2002; Ponglowhanap 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 -80ºC 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

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

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 (cm³) = [(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\textsuperscript{\textregistered}) (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\textsuperscript{\textregistered}) injected into the dog prostates resulted in no significant reduction in the gland and no pain/discomfort of the animals was clinically observed.

Acknowledgements

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References


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การศึกษาผลในด้านความเจ็บปวดและปริมาตรต่อมลูกหมากภายหลังการฉีดสารโบทูลินัมท็อกซิน ชนิดเอ เข้าสู่ต่อมลูกหมากสุนัข

ปัณฑิรา เที่ยงเธียรธรรม เกวลี ฉัตรดรงค์ ศุภวิวัฒน์ พงษ์เลาหพันธุ์

มีรายงานการศึกษาถึงความเป็นไปได้ในการฉีดสารโบทูลินัมท็อกซินเข้าสู่ต่อมลูกหมากเพื่อลดขนาดต่อมลูกหมากในคนและสัตว์ การศึกษานี้มีวัตถุประสงค์เพื่อประเมินผลของการฉีดสารโบทูลินัมท็อกซิน ชนิดเอ (BT-A) เข้าสู่ต่อมลูกหมางสุนัข โดยทำการประเมินในด้านความเครียด ความเจ็บปวด และปริมาตรต่อมลูกหมากหลังการฉีด การศึกษาในสุนัขนี้ถูกควบคุมให้ไม่ได้ทบทวนเจ้าหน้าที่จำนวน 6 ตัว มี BT-A จำนวน 100 ยูนิตเข้าสู่ต่อมลูกหมากผ่านทางผนังช่องท้องโดยใช้เครื่องอัลตราซาวน์ ทำการประเมินผลหลังการฉีด โดยทำการศึกษาแบบควบคุม ความเจ็บปวดจากการกินอาหารและการทาระเหมี่ยม จำนวนเม็ดเลือดขาวในกระแสเลือด และระดับคอร์ติซอล ก่อน (24 และ 12 ชั่วโมง) และหลัง (2 4 6 12 24 48 และ 72 ชั่วโมง) การฉีด BT-A และการฉีดสารโบทูลินัมท็อกซินผ่านทางอัลตราซาวน์ จำนวน (24 ชั่วโมง) และหลัง (30 60 90 วัน) การฉีด การศึกษานี้ได้ผ่านการพิจารณาจากคณะกรรมการการใช้สัตว์ทดลองจากสถาบันที่ทำการศึกษา จากการศึกษาพบว่าค่าคะแนนความเจ็บปวดจากการกินอาหารและการทาระเหมี่ยม จำนวนเม็ดเลือดขาวในกระแสเลือด และระดับคอร์ติซอลไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติระหว่างก่อนและหลังฉีดในทุกช่วงเวลาที่ทำการฉีดบันทึกตลอดระยะเวลาก่อนหลัง 72 ชั่วโมง นอกจากนี้ยังไม่พบการเปลี่ยนแปลงของปริมาตรต่อมลูกหมากระหว่างก่อนและหลังฉีด การศึกษาสรุปได้ว่าการฉีด BT-A ด้วยวิธีดังกล่าวไม่ก่อให้เกิดอาการเจ็บปวดหรือความเครียดต่อสุนัข และไม่มีการเปลี่ยนแปลงของขนาดต่อมลูกหมากในช่วงระยะเวลาที่ศึกษา

คําสั่งญาณ: โปรติเนียเน็กซีน สุนัข ต่อมลูกหมาก

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