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Pharmacology

Intra-articular botulinum toxin A (BoNT/A) for pain management in dogs with osteoarthritis secondary to hip dysplasia: A randomized controlled clinical trial

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ABSTRACT. The aim of this study was to evaluate the efficacy and safety of the intra-articular (IA) injection of botulinum toxin type A (BoNT/A) to the management of chronic pain in dogs. In a randomized, controlled, double-blinded study sixteen dogs with osteoarthritis secondary to hip dysplasia were distributed into two groups: 25 IU BoNT/A (BoNT) or saline solution (Control) was administered IA in each affected joint. All dogs received oral supplements (90 days) and carprofen (15 days). The dogs were assessed by a veterinarian on five occasions and the owner completed an assessment form at the same time (baseline to 90 days). The data were analyzed using unpaired-t test, Fisher's exact test, analysis of variance and the Tukey's test ($P<0.05$). There were no differences between groups in the veterinarian and owner assessments. Lower scores were observed in both groups during 90 days after IA therapy in the owner assessments ($P<0.001$). Compared with baseline, the Vet score was lower from 15–90 days after IA injection in the BoNT group, and at 15 and 30 days in the Control group ($P<0.001$). Both treatments were safe and reduced the clinical signs associated with hip osteoarthritis. However, IA BoNT/A (25 IU) did not provide better pain relief than the control treatment.

KEY WORDS: analgesia, botulinum toxin, canine, osteoarthritis, supplement

Osteoarthritis (OA) is one of the most common orthopedic diseases, which may be secondary to hip dysplasia (HD), mainly in medium to large-sized dogs. It is a painful chronic condition with a significant impact on quality of life, characterized by articular cartilage lesions and bone remodeling, with the presence of osteophytes and inflammation [23].

Non-steroidal anti-inflammatory drugs (NSAIDs) and oral nutraceuticals (e.g. glucosamine and chondroitin sulphate) are widely used for the symptomatic treatment of OA in dogs [3, 20]. However, long-term use of some NSAIDs may induce gastrointestinal, hepatic, and renal side effects [20, 28]. In addition, the efficacy of oral nutraceuticals in osteoarthritic dogs has been questioned. Data from a systematic review identified only weak evidence to support the use of nutraceuticals in canine OA [35]. Two placebo controlled clinical studies failed to detect significant improvements in subjective and objective measures using glucosamine and chondroitin when compared to placebo in osteoarthritic dogs [28, 31].

Intra-articular (IA) therapies are also clinical options for OA pain management [19]. In humans, there are an increasing number of clinical reports describing the use and effectiveness of IA botulinum toxin A (BoNT/A) for different types of OA [1, 18, 33]. BoNT/A represents an attractive therapy due to its prolonged duration of action, allowing a long interval between treatments, since the effects last around three to six months after a single IA application [22].

Although the precise mechanism by which BoNT/A produces analgesia is unknown, it has been suggested that this neurotoxin inhibits the release of a number of neurotransmitters such as glutamate, aspartate, substance P, and calcitonin gene-related peptide [2, 10, 11]. In addition, it has been demonstrated that BoNT/A inhibited inflammation and COX-2 expression in animal model of pain [8]. Chronic arthritis pain is a complex disease, involving peripheral and central sensitisation mechanisms. The release of neuropeptides from articular sensory nerve endings may amplify and perpetuate the joint pain [30]. Thus, when administered by IA route, BoNT/A may suppress the release of neuropeptides at the nociceptive nerve endings, directly reducing the peripheral

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sensitisation and, indirectly, the central sensitisation [24]. However, a recent study did not find any significant reduction in the synovial fluid concentrations of substance P and prostaglandin E2 from two to eight weeks after an IA BoNT/A injection in osteoarthritic dogs [13].

To date, in veterinary medicine few clinical studies have evaluated the antinociceptive efficacy of IA BoNT/A [12, 14]. Currently, only one randomized, placebo-controlled, double-blinded clinical trial on BoNT/A therapy for osteoarthritic dogs has been published [14].

To our knowledge there are no specific studies focused on IA BoNT/A therapy for the treatment of hip OA in dogs. Therefore, the present study aimed to evaluate the clinical efficacy and safety of IA administration of BoNT/A as an adjuvant to control chronic pain in dogs with OA induced by HD. The hypothesis was that the use of BoNT/A as an adjuvant would result in a better analgesic effect, compared to the control treatment.

MATERIALS AND METHODS

Animals

After obtaining informed consent, sixteen client-owned dogs were enrolled in the study. The study was approved by the Institutional Animal Care Committee (protocol 2041/2013 CEUA). Inclusion criteria were radiographic evidence of unilateral and/or bilateral OA secondary to HD (grade moderate to severe), signs of pain, according to the owners´ assessment, and at least two clinical signs of the disease (such as difficulty in: lying down or getting up from lying down, jumping or refusing to jump, going up or down stairs or lameness). Exclusion criteria included treatment with analgesic drug or nutraceuticals for at least four weeks before the initial evaluation. In addition, based on the clinical examination, dogs were excluded if they presented with lameness of both thoracic and pelvic limbs, showed neurological deficits, exhibited clinical signs of other orthopedic diseases and/or radiographic evidence of OA in any other joints, or had a history of recurrent gastritis or severe systemic diseases. If OA of another major joint was suspected clinically, additional radiographs were also made.

Radiographic evaluation

A radiographic examination was performed to confirm the presence of HD and determine the grade of HD according to the Orthopedic Foundation for Animals (OFA) classification (Orthopedic Foundation for animals) [29]. For the radiographic examination, the dogs were sedated intramuscularly (IM) with morphine (0.3 mg/kg; Dimorf Cristália, Itapira, Brazil), and anesthesia was induced with intravenous propofol (Propovan, Cristália, Itapira, Brazil) to effect. The radiological criteria of joint OA severities used in this study were based on the Kellgren-Lawrence criteria [21].

Study design

In a prospective, double-blinded, controlled clinical trial the dogs were randomly assigned to receive IA BoNT/A (BoNT) or 0.9% saline solution (Control). A random number generator (Research Randomizer, Computer software, http://www.randomizer.org/, Pennsylvania, PA, U.S.A.) was used to assign eight dogs to each of the two groups with block randomization in blocks of four animals. Prior to initiation of the treatment, all the animals were evaluated through physical and laboratory examinations (complete blood count and serum chemistry profile). A complete orthopedic evaluation was performed by one veterinary surgeon, who measured the clinical signs using an ordinal scoring system (Vet score, Table 1) modified from the article by Hielm-Björkman *et al.* [16]. In addition, the owners were asked to complete two descriptive questionnaires: Helsinki Chronic Pain Index (HCPI) [17] and Canine Brief Pain Inventory (CBPI), including the total pain scores (total CBPI score), pain severity scores (PSS) and pain interference scores (PIS) [6]. The veterinarian and owner assessments were repeated 15, 30, 60 and 90 days after IA treatment. Owners did not receive any specific training to perform the evaluations. The recommendation was that the same person complete the pain assessment questionnaires each time. Both veterinarian and owners were blinded to treatment allocation.

Treatments

Each dog in the BoNT group received an IA injection of 25 IU of BoNT/A (Dysport, Ipsen Pharmaceuticals, Dublin, U.K.) diluted in saline solution to a final volume of 0.5 m*l* in each affected joint. An equivalent volume of 0.9% saline solution was administered to the Control group. In addition, all the dogs were treated with one tablet of an oral nutraceutical (750–1,000 mg, q 12 hr, 180 days; Condroton, Vetnil, São Paulo, Brazil), and carprofen (2.2 mg/kg, q 12 hr, 15 days; Rymadil, Pfizer, São Paulo, Brazil). For the nutraceutical, the dosing regimen was 750 and 1,000 mg, respectively for dogs weighing 10–20 kg and 21–50 kg. The IA injections were performed by a veterinary surgeon. The dogs were positioned in lateral recumbency with the affected hip uppermost. The hair over the lateral aspect of the hip was clipped and the skin aseptically prepared. The arthrocentesis was guided by ultrasound, and the correct location of the needle (20 g) was confirmed through aspiration of synovial fluid. For the hip injection the dogs were anesthetized. After sedation with IM morphine (0.3 mg/kg), anesthesia was induced with IV propofol to effect, and maintained by isoflurane (Isoforine, Cristalia, Itapira, Brazil) with 100% oxygen using a rebreathing system (SAT 500, Takaoka, São Paulo, Brazil). Twenty-four hours after the IA injection, the owners were contacted by telephone for information on the behavior of the dog during this period, with questions on the presence of skin irritations or animal discomfort (such as: licking or biting the injection site, any increase in the degree of lameness, greater difficulty in getting up or lying down or the occurrence of vocalization). In addition, the owners were instructed to report the occurrence of behavioral changes and possible pain signals at any time.

Table 1. Clinical scoring system for assessing dogs (modified from Hielm-Björkman *et al.* [16]

Criterion	Grade	Clinical evaluation
Pain on manipulation	0	No signs of pain on palpation of the affected joint
	1	Slight signs of pain on palpation of the affected joint, the dog turns its head in recognition
	2	Moderate signs of pain on palpation of the affected joint, the dog pulls the limb as a defence reaction
	3	Severe signs of pain on palpation, the dog vocalizes or becomes aggressive
	4	The dog does not allow palpation
Lameness	0	Normal, no lameness
	1	Mild lameness, not very difficult to move
	2	Clear lameness, not moving freely
	3	Obvious lameness when walking
	4	Severe lameness preventing the dog from supporting weight on the affected limb
Ability to jump	0	Jumps normally
	1	Jumps with care
	2	Jumps with some difficulty
	3	Jumps or rises with great difficulty
	4	Does not try because of the difficulty/pain
Ability to climb stairs	0	Goes up and down the stairs normally
	1	Slightly careful, uses both paws successively
	2	Sometimes uses both feet at the same time, evidently does not move freely
	3	Goes up the stairs like a rabbit at all times, goes up the stairs with great difficulty
	4	Does not try to climb because of the difficulty/pain

Rescue analgesia

When dogs suffered severe pain the owners were instructed to give supplemental analgesics orally. During the initial 15-day period, tramadol (4 mg/kg, q 12 hr) was allowed as needed. After 15 days of IA injection, carprofen (2.2 mg/kg, q 12 hr) was given as rescue analgesia. The pain was considered severe if the dog exhibited the following signs: severe difficulty lying down or getting up; severe difficulty going.

Adverse effects

The occurrence of adverse effects was evaluated by the owners at home. If any dog exhibited signs of pain (e.g. more lameness, greater difficulty in getting up or lying down), the presence of bruising at the injection site, local muscle weakness (identified by the loss of tone and/or presence of tremors in the hip muscles and decreased ability to stand on the hind limbs), or systemic effects (fever, fatigue, gastrointestinal disorders) the recommendation was to inform the veterinarian of these events.

Outcome measures

The primary outcome measures were the CBPI and HCPI pain scores. Secondary outcome measures included: veterinarian assessment (Vet score), requirement for the rescue analgesia and adverse effects.

Statistical analysis

Assuming a baseline pain intensity of 5 on a 0–10 CBPI pain score, and a standard deviation (SD) of 1.1, it was estimated that a sample of at least eight subjects per group would provide an 80% chance (alpha level of 5%) of detecting a reduction in the CBPI scores of 30% compared to baseline (mean expected of 3.5 at the end of study). SD was estimated from a pilot study conducted in dogs with OA. The data were submitted to the Shapiro-Wilk and Kolmogorov-Smirnov normality tests to identify the distribution. For the variables weight, age and sex, the unpaired *t* test was used to compare the groups. The HCPI, CBPI and Vet scores were evaluated through ANOVA and the Tukey's test to compare differences between groups and differences over time within the same group. Additionally, treatment successes and failures were calculated in each group, as described by Brown *et al.* [5]. Treatment success was defined as a reduction ≥1 in PSS and ≥2 in PIS. To determine possible differences between the treatment groups, the two-tailed Fisher's exact test was used. The level of significance in all tests was 5%. The statistical analysis was performed using a statistical software package (GraphPad Instat 5.1, GraphPad Software Inc., San Diego, CA, U.S.A.).

RESULTS

Thirty-five dogs were initially screened to obtain 23 dogs eligible for inclusion in this study. Twelve dogs were excluded because of the following reasons: back pain associated with neurologic deficit and no hip OA (n=3), lack of OA in hip joints on radiographs (n=5), abnormal laboratory results (n=2) and multiple joints affected with OA (n=2). Of the 23 dogs initially enrolled in the study, 16 dogs completed the study. Seven dogs had to be excluded because four of the owners did not complete the evaluations post-treatment and three dogs died for reasons unrelated to the trial (pulmonary neoplasia, gastric dilatation-volvulus, and ehrlichiosis).

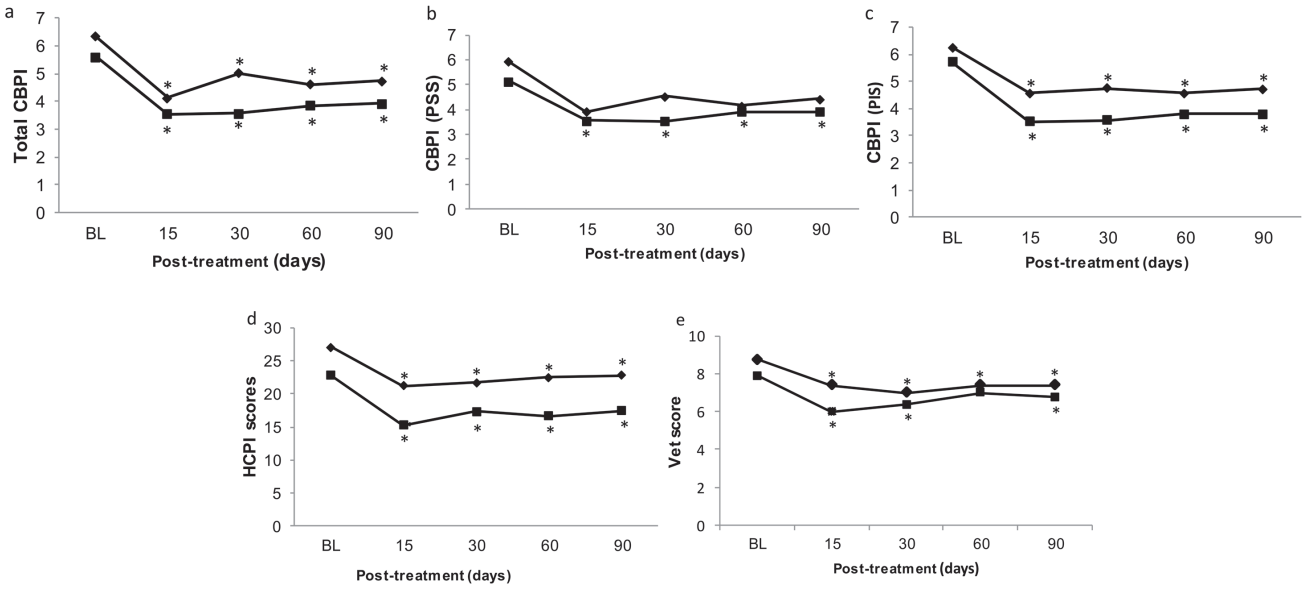

Fig. 1. Mean values of Canine Brief Pain Inventory (a), pain severity score (b) and pain interference score (c), Helsinki Chronic Pain Index (d) and Veterinarian score (e) prior to treatment (BL) and overtime in dogs treated with intra-articular botulinum toxin (diamond) or saline solution (square). *Significantly different from baseline values (Tukey's test, $P<0.05$).

Table 2. Baseline characteristics of the study population

Patient data		BoNT (n=8)	Control (n=8)	*P* (value)
Body weight (kg)[a)]		25.1 ± 12.7	24 ± 7.8	0.317
Age (years)[a)]		6.3 ± 3.9	4.6 ± 2.3	0.834
Male/Female[b)]		3/5	4/4	1.000
Body condition score[b)]				0.572
	Thin	0	1	
	Normal	6	6	
	Obese	1	0	
	Overweight	1	1	
Breeds[b)]				
	Border Collie	1	1	
	Labrador Retriever	1	3	
	Rottweiler	2	0	
	Lhasa Apso	1	0	
	Boxer	0	2	
	German Shepherd	1	0	
	Mixed-breed	2	2	
*Grade of HD[b)]				1.000
	Moderate	5	6	
	Severe	3	2	
HD status[b)]				1.000
	Bilaterally affected	8	7	
	Unilaterally affected	0	1	
Estimated duration of symptoms (months)[a)]		11.7 ± 6.4	11 ± 4.4	0.790
**Degree of OA[b)]				0.248
	Grade I	1	2	
	Grade II	3	4	
	Grade III	2	2	
	Grade IV	2	0	

a) Values expressed as mean ± SD. b) Number of dogs. *Grade of HD: Moderate=Significant subluxation, femoral head barely covered OA/remodeling/sclerosis along the femoral neck, head and acetabular rim; Severe=Severe subluxation, part or complete loss of coverage of the femoral head/ Large amounts of OA (based on the description provided on the OFA Web site). **Degree of OA: grade I=doubtful narrowing of joint space and possible osteophytic lipping; grade II=definite osteophytes and possible narrowing of joint space; grade III=multiple osteophytes, definite narrowing of joints space, some sclerosis and possible deformity of bone contour; grade IV=large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone contour.

Table 3. Number and percentage (%) of treatment successes and failures based on the success treatment criteria at 90 days post-treatment with intra-articular botulinum toxin (BoNT, n=8) or saline solution (Control, n=8) in osteoarthritic dogs

Success criteria	Success	Failure	*P* value
PSS ≥1; PIS ≥2			
BoNT	3 (37.5)	5 (62.5)	1.00
Control	4 (50.0)	4 (50.0)	

P value was derived with Fisher's test.

Baseline characteristics are descripted in Table 2. There were no significant differences between groups with respect to weight, age, grade of HD, degree of OA, and duration of symptoms.

The scores evaluated by the veterinarian or the owners did not significantly differ between groups at any time point ($P>0.05$). Compared to pre-treatment values, lower scores were observed in both groups from 15–90 days after IA injection in the CBPI (total score and PIS) and HCPI scores ($P<0.001$). The PSS scores were lower from 15–90 days compared to pre-treatment scores in the Control group ($P<0.001$). Compared with baseline, the Vet score was lower from 15–90 days after IA injection in the BoNT group, and at 15 and 30 days in the Control group ($P<0.001$) (Fig. 1).

The number and percentage of treatment successes and failures are summarized in Table 3. The success rate did not significantly differ between groups ($P=1.00$).

Rescue analgesia was not required during the evaluation period. Local adverse events, including pain in the first 24 hr after the IA injection occurred in four dogs in the BoNT group and one dog in the Control group. No dog exhibited adverse systemic effects or local muscle weakness.

DISCUSSION

Our results suggest that both IA BoNT/A and saline reduced the clinical signs of pain associated with OA secondary to HD. Neither veterinarian nor owner assessments were able to detect clear clinically relevant analgesic effects of IA BoNT/A added to conservative treatment. Therefore, our hypothesis of a superior analgesic effect following IA BoNT/A compared to the control treatment was denied.

There are several potential reasons for the results obtained. The combined use of drugs may have masked the potential analgesic benefits of BoNT/A. Despite limited and conflicting evidence, it has been suggested that the long-term use of oral nutraceuticals may reduce the clinical signs of OA and prevent articular degeneration [3]. Therefore, a slow onset of action, greater than 60 days, has been reported with oral glucosamine/condroitin in dogs [27]. On the other hand, carprofen, with a 10 to 14-day treatment period, has been shown to reduce the clinical signs of OA in a subjective clinical assessment [5, 7, 25] and in an objective analysis of the gait [5, 7] in osteoarthritic dogs. Thus, in the current study, the improvements in the owner and vet scores observed in both treatment groups at the initial evaluation period could be attributed at least in part to the carprofen. However, it is difficult to determine the exact period of time that carprofen could interfere in the results of this study. It has been suggested that a clinical response to carprofen can be maintained after the interruption of treatment [25, 26]. In addition, a clinical response to BoNT was expected from the first evaluation (15 days) after the IA injection. Data from a systematic review in humans suggested that the effects of BoNT/A start between two to 5 days, peak after 15 to 21 days, and last for approximately three to six months following IA injections [22]. In the current study, significant decreases in owner-subjetive pain assessments were detected during 90 days after IA BoNT/A injection, which is consistent with previously data found in canine OA studies [12, 14]. Nevertheless, in the same period of evaluation, a similar response was observed in the Control dogs. When comparing the two treatments there was no significant differences in any of the primary or the secondary outcome measures. Based on the CBPI data, the treatment was classified as a success for 37.5 and 50% of the BoNT and Control dogs, respectively, suggesting that IA BoNT/A was not superior to IA saline.

Placebo effects have been demonstrated in OA studies using client-owned dogs [6, 25]. Thus, it is possible that the results of the current trial could have been influenced by the caregiver placebo effect [9]. This effect represents the owners' perception of their dogs after the treatment. The owners may consider that the animals are improving and therefore reduce their estimation of pain. A recent study reported significant caregiver placebo effects in osteoarthritic dogs, with an occurrence of 56.9% of the time in the owner assessment [9]. Thus, given the small number of dogs included in this study, it is possible that the placebo effect may have masked the results. Although the owner-based questionnaires used in the current study have been previously validated, objective outcome measures such as kinetic force plates might have been used to obtain more reliable results. Nevertheless, conflicting results have been reported regarding the kinetic measurements for dogs suffering from OA. Two recent canine OA studies failed to detect a reliable correlation between CBPI and HCPI pain scores and kinetic evaluations [4, 34]. Moreover, Teixeira *et al.* [34] did not find significant differences between HD- affected and HD- free dogs using kinetic analysis.

The optimal therapeutic BoNT dosages are not well established. In humans, BoNT/A has been administered with doses ranging from 25 to 200 IU per joint [22]. In previous human trials, a significant decrease in pain scores has been reported following

IA injection of 100 IU in different types of OA [18, 32, 33]. Due to the lack of canine OA studies using IA BoNT/A during the experimental design of the current study, the dose at 25 IU was based on a preliminary study carried out in five osteoarthritic dogs [12]. Recently, a prospective double-blinded study compared the use of 30 IU of BoNT/A IA with a placebo in osteoarthritic dogs and detected no significant differences between groups in pain scores during the 12-week evaluation [14]. Although no dose response studies have been carried out in dogs, it is possible that the dosage administered in this study could be insufficient. Furthermore, until now, there is no comprehensive management index, including combinatons of BoNT/A with other medications.

In the present study, two pain score validated assessments (HCPI and CBPI) were used to obtain a more accurate overall impression of pain. The clinical signs of pain were also evaluated by a veterinary orthopedic surgeon experienced in the assessment of animals with hip HD, who has previously participated in other studies involving dogs with OA. In the current trial, no pain scale was sensitive enough to detect differences between groups. Other studies have also failed to demonstrate significant differences between groups using subjective pain scores for the evaluation of clinical signs in dogs with OA [14, 34]. Given the greater chance of placebo effects occurring in subjective measures [4], it would be ideal to repeat this study, including one group without treatment. A negative control group could lead to better understanding of the true impact of the two proposed treatments.

This study has some limitations. One potential reason for failure to demonstrate significant differences between groups can be attributed to the small sample size. The number of animals involved in the study was restricted due to the inclusion criteria and also the need for the owner's consent. Some of the owners refused participation in the study due to the need for general anesthesia to access the hip joint. In addition, a more accurate overall impression of pain could have been obtained by using objective outcome measures (e.g. reaction forces, accelerometer). Furthermore, the randomization of dogs between treatments resulted in groups that were not completely homogeneous. Despite significant differences were not detected between groups at baseline, the Control dogs presented lower pain scores and an inferior grade of OA. While 50% (4/8) of the dogs in the BoNT group exhibited a moderate to severe (III/IV) grade of OA, only 25% (2/8) of the dogs in the Control group exhibited a moderate grade (III) of OA. These findings suggest that the BoNT/A treated dogs presented greater disease severity at the beginning of the study, which could influence the response to treatment. Thus, these factors may explain in part the modest relief in pain symptoms following IA BoNT/A therapy.

Although rare, adverse events including fever, muscular weakness and fatigue have been reported in humans following IA BoNT/A therapy [18, 33]. In addition, complications of IA injections, such as joint infection, pain post-injection, and skin pigmentation may occur [19]. In the present study, no serious adverse events were noted. This result supports previous studies that reported minimal adverse effects following IA BoNT-A injection in dogs [12–15].

In conclusion, IA BoNT/A (25 UI) did not provide better pain relief than the control treatment. Further studies are required to determine the optimal dosage for IA-BoNT/A in dogs suffering from OA.

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