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Gonadotropin-releasing hormone immunization for the treatment of urethral sphincter mechanism incompetence in ovariectomized bitches

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ABSTRACT

We have investigated GnRH immunization for the treatment of urethral sphincter mechanism incompetence in ovariectomized bitches. It has been reported that decreasing LH secretion through the use of GnRH agonists temporarily restores continence in some bitches. Therefore, decreasing the circulating LH concentrations by immunizing against GnRH might temporarily maintain continence in incontinent dogs. Sixteen incontinent dogs given phenylpropanolamine (PPA) to control incontinence were recruited for this study. Eleven dogs were immunized against GnRH (novel treatment group) at week 0, and nine dogs were vaccinated again 4 weeks later. Five dogs (standard treatment group) were vaccinated with a placebo twice at 4-week intervals. PPA was discontinued in the novel treatment group 2 weeks after revaccination, and standard-treatment dogs were given PPA for the duration of the study. Blood samples were collected before each treatment and at 6, 8, 10, 12, 16, 20, and 24 weeks and owners recorded episodes of incontinence throughout the study. Ten of the eleven dogs in the novel treatment group experienced side effects as a result of vaccination; two of these dogs experienced more severe side effects after the first vaccination and were withdrawn from the study as a result. Of the nine dogs that completed the vaccination series, four dogs remained continent after PPA was discontinued. For these four dogs, there was no difference in incontinent episodes when they were given PPA versus treatment with the vaccine. All nine novel-treatment dogs developed a GnRH antibody titer and experienced a significant decrease in circulating LH concentrations. In conclusion, GnRH immunization was effective in maintaining continence in four of the nine incontinent ovariectomized dogs, and in these dogs, treatment with the vaccine was comparable with treatment with PPA.

1. Introduction

The development of urethral sphincter mechanism incompetence (USMI) in female dogs is a prevalent sequelae after ovariectomy or ovariohysterectomy (hereafter referred to as ovariectomy). Incidence of USMI are reported to be between 5.7% and 20% in ovariectomized bitches [1,2], whereas only 0% to 1% of intact bitches develop USMI [2]. After ovariectomy, urethral closure pressure decreases, even in bitches that remain continent [3]. However, urethral closure pressure is further reduced significantly in bitches with USMI [4].

Urethral closure pressure is normally maintained by sympathetic activation of α_1-adrenoreceptors in the urethral smooth muscle [5]. Therefore, the most common current method of treating USMI is with α-adrenergic agonists, specifically phenylpropanolamine (PPA). Phenylpropanolamine mimics the effect of catecholamines by activating α_1-adrenoreceptors in the urethral smooth muscle, effectively increasing urethral closure pressure and

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restoring continence. Unfortunately, PPA is not completely effective in the treatment of USMI [6,7]. In addition, PPA is not selective for α_1-adrenoreceptors within the urinary tract. Undesired vascular smooth muscle contraction from PPA administration elsewhere in the body, including blood vessels, has been shown to cause hypertension in humans and mice [8]; this adverse effect has also been reported in dogs [9]. Other reported adverse effects of PPA in dogs include anorexia, emesis and weight loss, lethargy and behavior changes, and proteinuria [9]. Another clinical difficulty with the use of PPA is the 4-hour half-life [10] that requires dosing every 8 to 24 hours to maintain therapeutically effective urethral closure. This frequent administration can be frustrating for owners because treatment must be continued for the rest of the dog's life.

Ovariectomy results in elevated circulating concentrations of pituitary LH because there is no gonadal negative feedback. LH receptors are present throughout the canine urinary tract [11–13] and it has been postulated that elevated gonadotropins may contribute to the development of USMI [14]. Treatment of bitches with long-acting GnRH agonists downregulates LH secretion for prolonged time periods [15] and temporarily restores continence to incontinent bitches for varying durations, ranging from 50 to 738 days in one study [14] and 70 to 575 days in another [16]. Similar to PPA, GnRH agonists are not completely effective for the treatment of USMI [14,16]. However, unlike PPA, no adverse effects to GnRH agonists have been reported.

Unfortunately, GnRH agonist availability is limited in the United States. Although there are GnRH agonists available that are approved for the treatment of human diseases, such as prostate cancer, they are costly and not financially feasible for a pet owner to consider [17–19]. To date, deslorelin acetate (Suprelorin, Virbac Animal Health, Fort Worth, TX, USA) is the only GnRH agonist that has been developed for use in domestic animals [20]; however, it is currently available in the United States only for the treatment of adrenal disease in ferrets and extra-label use is explicitly prohibited [21].

There are other methods reported that temporarily decrease LH concentrations and therefore may also treat USMI, such as immunization against GnRH. Immunization against GnRH elicits the synthesis of GnRH-neutralizing antibodies, which prevent GnRH from binding to GnRH receptors and consequently prevent the synthesis of LH [22]. In 2004, a commercial GnRH vaccine was launched in the United States (Canine Gonadotropin Releasing Factor Immunotherapeutic; Pfizer Animal Health, Exton, PA, USA). This vaccine was labeled for the treatment of benign prostatic hyperplasia in intact male dogs with recommended revaccination every 6 months for effective treatment. It has also been shown to decrease testosterone concentrations in intact male dogs for approximately 20 weeks [23] and to safely terminate pregnancy in bitches [24].

The objectives of this study were to determine: (a) whether GnRH immunization will maintain continence in incontinent ovariectomized bitches; and (b) whether GnRH immunization controls USMI as effectively as PPA. It was hypothesized that GnRH immunization would effectively maintain continence for a prolonged duration and that it would be as effective as PPA for the treatment of USMI.

2. Materials and methods

2.1. Animals and vaccination

Sixteen privately owned ovariectomized bitches were enrolled at Oregon State University's Veterinary Teaching Hospital under the oversight of Institutional Animal Care and Use Committee for this study. A diagnosis of incontinence after ovariectomy was confirmed using veterinary medical records. At the time the bitches that were recruited for the study were all being treated with PPA (Proin; PRN Pharmacal, Pensacola, FL, USA) to maintain continence. Dogs received varying doses of PPA as prescribed by their regular veterinarian; doses corresponding to each dog are summarized in Table 1. Clinical health was confirm in all dogs by a complete blood count, biochemistry panel, urinalysis, and urine culture at the beginning and end of the study.

Novel-treatment dogs ($n = 11$) received 1 mL Canine Gonadotropin Releasing Factor Immunotherapeutic subcutaneously over the lateral thorax and were reimmunized 4 weeks later. Standard-treatment dogs ($n = 5$) received 1 mL saline over the lateral thorax and were treated again 4 weeks later. Animals were closely monitored by their owners for adverse reactions. One novel-treatment dog developed tachypnea for 24 hours after initial vaccination and another novel-treatment dog demonstrated impaired movement because of soreness for 1 week after initial vaccination. These two dogs did not receive a second vaccination and were excluded from further study.

2.2. Study design and sample collection

Venous blood samples were collected from standard- and novel-treatment dogs before each treatment (0 and 4 weeks) and again at 6, 8, 10, 12, 16, 20, and 24 weeks after initial vaccination. Blood samples were divided into Vacutainer clot tubes (02-685-A, Fisher Scientific Co., Waltham, MA, USA) to obtain serum and Vacutainer EDTA tubes (02-683-99 A, Fisher Scientific Co.) to obtain plasma. After centrifugation, serum and plasma were separated and frozen at $-20\ ^{\circ}C$ until analysis.

Use of PPA was discontinued in novel-treatment dogs 2 weeks after the second vaccination, and standard-treatment dogs continued to receive PPA for the duration of the study. All novel-treatment dogs were not given PPA for at least 1 week after PPA was discontinued; when the dog became incontinent again, PPA administration was resumed.

Owners reported the frequency of incontinent episodes before any treatment for incontinence was initiated and they also recorded all episodes of incontinence for the duration of the study. For novel-treatment dogs that maintained continence after PPA discontinuation, comparisons were made between PPA treatment and treatment with the vaccine. The frequency of incontinent episodes on initial USMI diagnosis (before any treatment was initiated) during treatment with PPA (week 0 through week 6 of the