A NEW DRUG DELIVERY FORMULATION OF GnRH ANALOGUE DESLORELIN FOR CONTRACEPTION IN CANINES: A SIGNIFICANT STEP FORWARD IN ANIMAL WELFARE

FORMULASI BARU PENDISTRIBUSIAN OBAT AGONIS GnRH DESLORELIN UNTUK KONTRASEPSI ANJING: SUATU LANGKAH KEDEPAN DALAM KESEJAHTERAAN HEWAN

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ABSTRACT

The effect of chronic treatment with a slow release implant containing GnRH agonist deslorelin on pituitary and testicular function was studied in Indonesian mature male dogs. Eight dogs were implanted with 10 mg deslorelin implant (group 1). Group 2 (n=3) were control dogs and given an implant without deslorelin (placebo implant). Plasma testosterone concentrations were undetectable on day 30 after implantation in treated dogs. Testes volume dropped to 35% after 13 weeks and ejaculate could not be obtained 4 weeks after implantation. Histological assessment of the testes and prostate gland showed atrophy of the seminaliferous tubules. Spermatogenesis was absent and the glandular epithelium of the prostate was atrophic and non-secretory. This study demonstrates that the drug delivery formulation containing deslorelin was effective in long term suppression of reproductive function in male dogs. There were no adverse side effects seen in any of the dogs treated.

Key words: GnRH agonist, deslorelin, testosterone, spermatogenesis

ABSTRAK


Kata kunci: Agonis GnRH, deslorelin, tesosteron, spermatogenesis.
INTRODUCTION

Humane and efficient population control of dogs remains a priority in many countries. Most of the research concerned with reproductive control in dogs has been directed towards developing a contraceptive for use in females. Surgical sterilization is the only commonly used method of sterilization for male (Pintado, 1989). However, this method is costly, and requires supervision by professional staff and is not practical for large stray dog populations.

The administration of GnRH analogues has been shown to be effective in suppressing testosterone secretion and spermatogenesis in a wide range of species including the dogs (Vickery et al., 1984; Parmar et al., 1993; Tashra et al., 1996). To be effective it must be administered over long periods of time. A delivery system which is easy to use, safe, bio-compatible, provides long-term release of sufficient amounts of GnRH agonist and is cost effective has been developed by Peptech Animal Health, a Sydney based biotechnology company. This proprietary technology meets the criteria for long-term contraception of dogs.

This was an important animal welfare project for Indonesia, aimed at providing an alternative to surgical castration for the contraception of dogs.

MATERIALS AND METHODS

Animals. Eight male Indonesian local dogs ranging in age from 2 to 3 years and weight ranging from 5 to 12 kg were used in this experiment. The animals were housed indoors at Veterinary Clinic Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia. During the day they were outdoors for 1 to 2 hours. Food was provided once daily in amounts adequate to maintain body mass and had access to water ad libitum. The dogs were vaccinated against Distemper, Hepatitis and Parovirus with CANGEN® DH12P (Vickie Laboratories, NZ, Ltd, Auckland) and dewormed using Drontal tablets (Sayer, Australia Ltd). The dogs were randomly assigned into two groups. Group 1 dogs (n=8) each received a 10 mg deslorelin implanted subcutaneously. Group 2 dogs (n=3) each received an implant without deslorelin (placebo implant).

Semen was collected at weekly intervals by hand manipulation without a teaser bitch, as described by Seager (1986). Only the second, sperm-rich fraction was collected. Immediately after collection, sperm concentration was determined by haemocytometer count and motility by microscopic examination of a dog of semen. A sigmoid/equin preparation was made for subsequent assessment of the percentages of live spermatozoa and the percentage of abnormal spermatozoa. A total of 100 spermatozoa were counted on each slide.

Testes volume was estimated using calipers as described by Love et al., (1991). The length, width and height of both testes were measured. Each measurement was taken 3 times and the values averaged to give the recorded measurement. The formula for an ellipsoid (Volume = 4/3πabc; a= length/2, b= width/2, c= height/2) was used to estimate testicular volume.

Blood samples were collected at weekly interval for 3 weeks before insertion of the implants, to determine the normal physiological concentrations and pattern of release of testosterone. After insertion of the implants, blood was sampled at weekly intervals for 3 weeks, then twice a month for the duration of the experiment. Blood samples were placed into lithium heparin tubes and immediately centrifuged at 2000 rpm for 10 minutes at 4°C. The plasma was separated and stored in 2 separate 5 ml plastic vials at -20°C until assayed.

Two dogs from group 1 and one dog from group 2 were sacrificed at 3 months and again at 8 months after implantation to assess histology of testes, epididymis and prostates of the dogs. The remaining 4 treated dogs will be monitored until they recover normal fertility. Immediately after the animals had been killed, tissue samples from testes, prostate and epididymis were collected and fixed in Bouin’s solution for 24 hours. The fixed tissues were then placed in 70% alcohol for 48 hours and processed for histology. Each specimen was embedded in paraffin wax and 5um sections were cut and stained with haematoxylin/eosin.

Deslorelin implant. The GnRH agonist, deslorelin (D-Trp7-Pro2-des-Gly4-LHRH ethylamide), was prepared and supplied as implants by Peptech Animal Health Pty Limited (Sydney, 23
Australia) that were 0.23 x 15.2 mm and contained 10 mg deslorelin. Implants were manufactured by extrusion of deslorelin with a matrix consisting principally of low-melting point lipids and biological surfactant. In a real time dissolution system, these implants released doses of > 1 µg/day for extended periods. Implants were prepackaged in syringes equipped with a 13 gauge needle and were injected subcutaneously in the neck between the shoulder blades under aseptic conditions.

**Testosterone assay.** Plasma testosterone was measured using radioimmunoassay (RIA) as previously described (Junaidi et al., 1998). The limit of detection was 0.6 ± 0.2 ng/ml.

Statistical analyses. Hormone concentrations of testosterone was shown as mean ± SEM. Differences in mean semen volume, testicular volume, testosterone concentrations within and between animals were evaluated by ANOVA, followed by pairwise comparisons of means TUKEYS (HSD) using Statistics version 4.1 © 1994, (Analytical Software). The level of significance was set at \( P < 0.05 \).

**RESULTS**

The insertion of the deslorelin implants resulted in a marked decrease in the plasma concentration of testosterone below pretreatment values after 15 days (Fig. 1). The concentrations fell to below the sensitivity of the assay (0.6 ng/ml) by 23 days after implantation. Plasma testosterone concentrations remained completely suppressed and undetectable for 34 weeks. At present, remaining treated dogs are still under observation for the recovery phase. There was no significant change in the plasma testosterone concentrations in control dogs over the period of blood sampling (Fig. 1).

After 4 weeks of treatment, no ejaculates were produced (Fig. 2). Before the cessation of ejaculation, reductions in motility were observed.

The testicular volume of the dogs prior to and following injection of the deslorelin implant are presented in Fig. 3. The mean testicular volume of each dog dropped significantly (\( P < 0.05 \)) after 8 weeks of implantation, with the low volume being maintained for 34 weeks.

Histological assessment of the testes and prostate after 3 and 8 months of implantation showed atrophy and aspermato genesis of the seminiferous tubules and the glandular epithelium.

![Fig 1. Mean ± SE plasma testosterone concentrations in dogs (group 1) following implantation with 10 mg GnRH agonist deslorelin (●) and in control dogs (group 2) following implantation with placebo implants (□).](image1)

![Fig 2. Mean ± SE semen volume in male dogs following implantation of GnRH agonist deslorelin (●) and in control dogs implanted with placebo implant (□).](image2)
Fig. 3. Mean ± SE testicular volume in male dogs following implantation of GnRH agonist deslorelin (□) and in control dogs implanted with placebo implant (●).

No sign of inflammatory reaction was seen at the site of implantation and there was no associated oedema following implantation. There were no adverse side effects seen in any of the dogs.

DISCUSSION

Plasma concentrations of testosterone fell progressively and became undetectable after 30 days. This progression was also found in the previous studies in dogs using similar implant of GnRH agonist deslorelin (Jauanid et al., 1998; Trigg et al., 2001). The decrease in plasma testosterone concentrations was followed by a progressive decrease in the ejaculate volume, accompanied by a decline in the motility and maturity of spermatozoa in the ejaculate. In the dog, testosterone controls the prostate secretion and is also required for maintenance of spermatogenesis (Gilbert and Bonu, 1987). The suppression of testosterone secretion from the testes may due to a loss of testicular LH receptors in response to the continuous dose of deslorelin. Dube et al. (1987) found that an increase in endogenous LH after injection GnRH agonist is followed by a marked and sustained loss of testicular LH receptors and a marked atrophy of Leydig cells. Moreover, McLachlan et al. (1995) explained that LH receptors are only found on Leydig cells and the action of LH is through the stimulation of testosterone secretion by Leydig cells. The decrease in testosterone observed in dogs treated chronically with GnRH agonist is likely to be due to the decrease in plasma concentrations of LH and possible reduction in testicular LH receptor. Histological findings of the seminiferous tubules and prostate were described by (Cavite et al., 1988) in dogs after 91 days of treatment with 50 μg/GnRH agonist (D-Trp6 LHRH)/kg. The lack of germ cells in the lumen of seminiferous tubules in our study and the apparent increase in Sertoli cell number is indicative of tubule atrophy (Dube et al., 1987).

Chronic stimulation of gonadotropes with a GnRH agonist causes pituitary desensitization with a complete loss of bioactive LH (St-Arnaud R et al., 1986). Jauanid et al. (1998) and Trigg et al. (2001) found that the long-term effect of the GnRH agonist deslorelin in the male dogs showed full down-regulation and desensitization of the anterior pituitary gland, and explains the loss of testicular function. At the testicular level, the absence of stimulation by LH would cause a reduction in testosterone secretion which can explain Leydig-cell atrophy and would be secondarily responsible for the atrophy of the seminiferous tubules deprived of androgens. The effect of the treatment is expected to be fully reversible (Jauanid et al., 1997 and Trigg et al., 2001).

Implantation of a slow release implant containing 10 μg of the GnRH agonist deslorelin in the dogs is effective in long-term suppression of the reproductive function in male dogs and there were no adverse side effects seen in any of the dogs treated.

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REFERENCES


