Leuprolide acetate treatment of adrenocortical disease in ferrets

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Objective—To determine the effects of leuprolide acetate, a long-acting gonadotropin-releasing hormone analog, in ferrets with adrenocortical diseases.

Design—Case series.

Animals—20 ferrets with adrenocortical disease diagnosed on the basis of clinical signs and plasma sex hormone concentrations.

Procedure—Ferrets were treated with leuprolide (100 µg, IM, once), and plasma hormone concentrations were measured before and 3 to 6 weeks after treatment.

Results—Leuprolide treatment resulted in significant reductions in plasma estradiol, 17 α-hydroxyprogesterone, androstenedione, and dehydroepiandrosterone concentrations and eliminated or reduced clinical signs associated with adrenocortical disease. Decreases in vulvar swelling, pruritus, and undesirable sexual behaviors and aggression were evident 14 days after treatment; hair regrowth was evident by 4 weeks after treatment. The response to treatment was transitory, and clinical signs recurred in all ferrets. Mean ± SEM time to recurrence was 3.7 ± 0.4 months (range, 1.5 to 8 months).

Conclusions and Clinical Relevance—Results suggest that leuprolide can be safely used to temporarily eliminate clinical signs and reduce sex hormone concentrations in ferrets with adrenocortical diseases. However, the safety of long-term leuprolide use in ferrets has not been investigated, and the long-term effects of leuprolide in ferrets with nodular adrenal gland hyperplasia or adrenal gland tumors are unknown. (*J Am Vet Med Assoc* 2001;218:1272–1274)

Adrenocortical diseases (ACD) including nodular hyperplasia, adrenocortical adenoma, and adrenocortical adenocarcinoma are common in neutered middle-aged to older ferrets. The adrenal tissues of affected ferrets produce a variety of sex hormones such as estradiol, **17 α-hydroxyprogesterone (17-OHP)**, androstenedione, and **dehydroepiandrosterone sulfate (DHEA)**. The major clinical signs attributable to these diseases are alopecia and a swollen vulva in females. Pruritus, muscle atrophy, hind limb weakness, and sexual activity or aggression are seen less frequently. Males can develop prostatic cysts and urethral obstruction. With some adrenocortical diseases, metastases, although rare, may develop, and bone marrow suppression can be a fatal sequela to chronic exposure to these hormones, especially estrogen.[1-4]

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The high prevalence of ACD in pet ferrets is associated with neutering at an early age and may be attributable to chronic stimulation of the adrenal cortex by the gonadotropins **follicle-stimulating hormone (FSH)** and **luteinizing hormone (LH)**.[1,5] Abnormally long photoperiods associated with indoor housing of pet ferrets is also thought to contribute to the pathogenesis. Light cycles > 8 hours long have been shown to stimulate production of **gonadotropin-releasing hormone (GnRH)** and LH in ferrets.[6] Suppression of these gonadotropins may limit adrenal gland hyperplasia, tumor growth, and subsequent sex hormone production.

Administration of GnRH analogs at pharmacologic doses suppresses production and release of gonadotropins by downregulating GnRH receptors at the pituitary.[7] The purpose of the study reported here was to determine the clinical effects of administration of leuprolide acetate, a long-acting GnRH analog that results in prolonged suppression of pituitary gonadotropin release, in ferrets with ACD.

Materials and Methods

Twenty client-owned ferrets with ACD were used in the study. Severity of ACD and duration of clinical signs varied, and no attempts were made to differentiate nodular hyperplasia from adrenocortical adenoma from adrenocortical adenocarcinoma. Ferrets ranged from 3 to 7 years old (median, 5 years). Fourteen ferrets were female, and 6 were male; all had been castrated or spayed before 2 months of age.

In all ferrets, the diagnosis of ACD had been made on the basis of clinical signs and plasma hormone concentrations. Clinical abnormalities included alopecia, pruritus, a swollen vulva in females, and an increase in sexual behaviors and aggression.

Ferrets were anesthetized with isoflurane administered via a face mask, and a complete physical examination, including percutaneous palpation of the adrenal glands and adrenal gland ultrasonography, was performed. Blood samples were collected, and leuprolide[a] (100 µg, IM) was administered. For administration, leuprolide was diluted to a concentration of 100 µg/0.2 ml with 7.5 ml of the accompanying diluent and stored in individual injection vials at –4 C. Ferrets weighed between 0.66 and 1 kg (1.45 and 2.2 lb); therefore, the leuprolide dose was 100 to 150 µg/kg (45 to 68 µg/lb). Blood samples were submitted for determination of Hct, serum glucose and urea nitrogen concentrations, serum **alanine aminotransferase (ALT)** activity, and plasma hormone concentrations. Plasma samples were stored at –70 C until shipped to the laboratory[b] for analysis.

Ferrets were reexamined 3 (6 ferrets), 4 (5), 5 (5), or 6 (4) weeks after leuprolide treatment, and follow-up blood samples were collected. In addition, owners were contacted by telephone every 2 to 4 weeks.

Plasma estradiol, androstenedione, 17-OHP, and DHEA concentrations were measured by use of commercially avail-

able radioimmunoassays validated for use in ferrets. Concentrations were compared with established reference range values for ferrets (estradiol, 30 to 180 pmol/L; androstenedione, 0 to 15 nmol/L; 17-OHP, 0 to 0.8 nmol/L; and DHEA, 0 to 28 nmol/L).[1,2]

Statistical analyses—A repeated-measures analysis[c] was used to compare plasma hormone concentrations before and after treatment with leuprolide. Plasma hormone concentrations 3 to 6 weeks after treatment were combined and compared with plasma hormone concentrations before treatment. Differences in individual least-squares means for pretreatment and posttreatment hormone concentrations were evaluated by use of the **least significant difference (LSD)**. In addition, mean values were tested against the upper reference limit with a *t*-test. Data are given as mean ± SEM. A value of $P < 0.05$ was considered significant.

Results

All ferrets had clinical signs consistent with a diagnosis of ACD prior to treatment with leuprolide. The most consistent findings were bilaterally symmetric truncal alopecia and vulvar swelling in females of at least 2 months' duration. None of the ferrets had palpably enlarged adrenal glands. Adrenal gland ultrasonography was performed prior to leuprolide treatment in 12 ferrets, and the adrenal glands were enlarged in 8. Adrenal gland diameter ranged from 3 to 12 mm (reference range, < 5.5 mm). Hematocrit, serum glucose and urea nitrogen concentrations, and serum ALT activity were within reference ranges before and after treatment with leuprolide.

Owners did not report any adverse effects associated with leuprolide treatment. During the first 10 days after leuprolide treatment, most ferrets were more active, and owners reported that the ferrets appeared to feel better. Six ferrets had pruritus prior to treatment with leuprolide, and in all 6, the severity of pruritus was greatly reduced by 2 weeks after treatment. Five ferrets exhibited sexual behaviors or increased aggression prior to leuprolide treatment, and in all 5, these behaviors were decreased or eliminated by 2 weeks after treatment. Fourteen ferrets had swollen vulvas prior to leuprolide treatment; in 11 of the 14, turgidity of the vulva had decreased by 10 to 14 days after treatment, and the vulva appeared normal by 6 weeks after treatment. Most ferrets began to regrow hair 4 weeks after treatment with leuprolide. Fourteen of 17 ferrets with alopecia had 90 to 100% hair regrowth by 8 weeks after treatment. The remaining 3 ferrets had 70 to 80% regrowth of hair by 8 weeks and maintained a thin pelage thereafter. Eight ferrets had thin incomplete regrowth of the tail hair.

Clinical signs recurred in all ferrets following treatment with leuprolide. Mean ± SEM time to recurrence was 3.7 ± 0.4 months (range, 1.5 to 8 months). Female ferrets had the longest and the shortest times to recurrence of clinical signs.

None of the ferrets died before clinical signs of ACD recurred. Five ferrets required additional evaluation and treatment for concurrent diseases. Two ferrets had an insulinoma, 2 ferrets had cardiomyopathy, and 1 ferret had renal disease. One ferret developed a large inoperable right adrenal gland adenocarcinoma during the study; a biopsy was performed after clinical signs recurred. The tumor was palpated 5 months after leuprolide treatment and was 50 mm in diameter at the time of surgery. Diameter of the right adrenal gland, measured ultrasonographically, before and 5 weeks after leuprolide treatment was 8 mm, indicating that all tumor growth had occurred > 5 weeks after treatment. None of the 12 ferrets in which adrenal gland ultrasonography was performed had an increase in adrenal gland diameter 3 to 6 weeks after leuprolide treatment, but additional follow-up ultrasonography > 6 weeks after treatment was not performed.

Plasma estradiol, 17-OHP, androstenedione, and DHEA concentrations were measured in all ferrets before and 3 to 6 weeks after leuprolide treatment. For all ferrets, plasma concentration of at least 1 hormone was greater than the upper reference limit prior to treatment. Mean ± SEM estradiol concentration before treatment (181.6 ± 12.6 pmol/L) was similar to the upper reference limit (180 pmol/L) and significantly ($P < 0.001$) higher than mean concentration measured 3 to 6 weeks after treatment (94.9 ± 12.6 pmol/L). Mean 17-OHP concentration before treatment (2.31 ± 0.29 nmol/L) was significantly ($P < 0.001$) greater than the upper reference limit (0.8 nmol/L) and the mean concentration measured 3 to 6 weeks after treatment with leuprolide (1.18 ± 0.29 nmol/L). Mean androstenedione concentration before treatment (86.0 ± 13.7 nmol/L) was significantly ($P < 0.001$) greater than the upper reference limit (15 nmol/L) and the mean concentration measured 3 to 6 weeks after treatment (31.8 ± 14.1 nmol/L. Mean DHEA concentration before treatment (22.9 ± 3.6 nmol/L) was similar to the upper reference limit (28 nmol/L) and significantly ($P < 0.007$) higher than mean concentration measured 3 to 6 weeks after treatment (10.4 ± 3.4 nmol/L).

Discussion

In the present study, leuprolide treatment of ferrets with ACD resulted in significant reductions in plasma estradiol, 17-OHP, androstenedione, and DHEA concentrations and eliminated or reduced clinical signs associated with these diseases. No adverse effects were identified; however, effects were only temporary in that clinical signs recurred in all ferrets, typically by 3 months after treatment. The duration of clinical effect ranged from 1.5 to 8 months, and similar wide ranges in duration of effect have been reported for another GnRH analog, deslorelin, in dogs, cats, and cattle.[8-10] The reason for this variable effectiveness is unknown but is probably related to dose and individual sensitivity to the GnRH analog. It is possible that repeated injections may suppress clinical signs for prolonged periods; however, the safety of long-term leuprolide use in ferrets has not been investigated, and the long-term effects on adrenal gland hyperplasia or growth of adrenal gland tumors are unknown.

No attempt was made to differentiate nodular hyperplasia from adrenocortical adenoma or adrenocortical adenocarcinoma in the present study, and it is possible that these different adrenal lesions respond in different ways to leuprolide treatment. Previous studies of adrenal lesions in ferrets with ACD have shown that approximately 56% have nodular hyperplasia, 16%

have adrenocortical adenoma, and 26% have adrenocortical adenocarcinoma,[4] and it is likely that the 20 ferrets in the present study had a similar distribution of adrenal lesions. All ferrets had similar clinical responses to leuprolide treatment; however, leuprolide's effectiveness in ferrets with specific adrenal gland abnormalities needs to be evaluated further.

Leuprolide is a potent long-acting GnRH agonist that effectively suppresses production of the pituitary gonadotropins FSH and LH. A sufficiently high concentration of leuprolide for a prolonged period desensitizes the pituitary gland to native GnRH stimulation, with subsequent suppression of gonadal sex hormone production. In gonadectomized ferrets, leuprolide may have a similar effect on adrenal gland production of sex hormones.

The high prevalence of ACD in pet ferrets is associated with neutering at an early age and may be attributable to chronic stimulation of the adrenal cortex by FSH and LH.[1,5] In spayed ferrets, plasma FSH and LH are high,[11] and in spayed and castrated dogs, serum LH and FSH concentrations are 10 times those in sexually intact dogs.[12] Abnormally long photoperiods associated with indoor housing of pet ferrets may also contribute to the pathogenesis of ACD, as light cycles > 8 hours long have been shown to stimulate GnRH and LH production in ferrets.[6] It has been shown that humans and mice have LH receptors in the adrenal glands and in adrenal gland tumors, and it is has been suggested that these cells with LH receptors are steroidogenic.[13,14] Results of preliminary studies performed by 1 of the authors (RAW) also indicate that adrenal gland tumors in ferrets have LH receptors. Mice have been known to develop adrenal gland tumors following castration,[15] and LH is known to cause adrenocortical tumorigenesis in castrated mice.[14] Suppression of these gonadotropins may, therefore, limit adrenal gland hyperplasia, tumor growth, and subsequent sex hormone production in ferrets with ACD.

At this time, surgical removal is the only curative treatment for ferrets with adrenal gland tumors.[2-4] Results of the present study, however, suggest that leuprolide may be used as a safe short-term alternative to surgical treatment. Leuprolide may be especially helpful in old or medically compromised ferrets that may not be able to undergo anesthesia and surgery.

[a]Lupron Depot (3.75 mg), TAP Pharmaceuticals Inc, Deerfield, Ill.

[b]Clinical Endocrinology Service, University of Tennessee, Knoxville, Tenn.

[c]PROC MIXED, SAS Institute Inc, Cary, NC.

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