

CUSHING'S DISEASE IN DOGS: RECOMMENDED DIAGNOSTIC PROTOCOLS AND TREATMENT OPTIONS

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Definitions of Typical, Atypical, and Pseudo-Cushing's Syndrome

Hyperadrenocorticism or Cushing's syndrome is one of the most common endocrine disorders in middle-aged and older dogs. Presently, there are three clinical variants of this disorder: typical, atypical, and pseudo-Cushing's disease.

What is Classical Typical Cushing's Syndrome?

Classical or typical hyperadrenocorticism is a syndrome caused by excess secretion of cortisol from the adrenal gland(s). Approximately 80 to 85% of cases of spontaneous hyperadrenocorticism in both dogs and cats are due to pituitary-dependent hyperadrenocorticism, with the remainder due to a functional adrenocortical tumor. Clinical signs include polyuria and polydipsia, polyphagia, panting, pot-bellied appearance, and bilateral alopecia. Common biochemical profile and urinalysis abnormalities include serum alkaline phosphatase and cholesterol elevations, low urine specific gravity, and proteinuria.

The diagnosis is confirmed by standard testing protocols, including the ACTH stimulation test, low-dose dexamethasone suppression test, and urine cortisol: creatinine ratio. In general, we would like 2 of these 3 tests to be positive in order to confirm the diagnosis. Most dogs with typical Cushing's disease can be treated successfully with either mitotane or trilostane.

What is Atypical Cushing's Disease?

Some dogs with classic signs of Cushing's disease and typical hematological and biochemical findings have a normal cortisol response to ACTH administration or low-dose dexamethasone suppression testing and normal urine cortisol:creatinine ratio. It has been suggested that cases of atypical Cushing's disease may have a derangement of the steroid production pathway and that some of the precursors of cortisol, such as 17-OH progesterone may be abnormally increased. Therefore, the term atypical Cushing's disease refers to hyperadrenocorticism caused by increased levels of intermediate adrenal steroids (documented by ACTH stimulation) that frequently are referred to as "sex steroids."

Dogs with this atypical disorder may respond to mitotane or trilostane, similarly to dogs with typical or classical Cushing's disease. Anecdotal reports suggest that melatonin and flax seed oil with lignans may also be occasionally effective for other dogs with atypical Cushing's disease.

What is Pseudo-Cushing's Disease?

Pseudo-Cushing's disease is more commonly known as Alopecia X or adrenal-hyperplasia syndrome, and is associated with intense hyperpigmentation of alopecic areas principally recognized in young "plush-coated" breeds such as the Pomeranian, Miniature poodle, Samoyed, and Alaskan malamute.

Alopecia X has been previously termed growth-hormone responsive alopecia, adrenal sex hormone imbalance, castration responsive dermatosis, and congenital adrenal hyperplasia. These names reflect the lack of clear understanding of the pathogenesis, although recent data suggest this condition is associated with abnormal steroidogenesis and thought to be a mild form of pituitary-dependent hyperadrenocorticism; In Pomeranians a late-onset 21-hydroxylase deficiency has been proposed. Alopecia is often first observed between 1 and 5 years of age. Symmetrical alopecia affects the caudal thighs, the trunk, perineum or neck, and often there are changes in coat color. There are no other clinical signs and no biochemical or urinalysis changes.

The measurement of 17-hydroxyprogesterone before and after administration of ACTH has been recommended in the investigation of dogs with Alopecia X as an indicator of abnormal steroidogenesis. Concentrations of 17-hydroxyprogesterone were elevated post-ACTH stimulation in all 31 affected Pomeranians in one European clinical study. While an elevation of 17-hydroxyprogesterone in part supports a diagnosis of Pseudo-Cushing's disease, following exclusion of hypothyroidism and hyperadrenocorticism, the role of the hormone in the development of the hair loss is unclear. Dogs with this disorder may respond to castration, methyltestosterone, melatonin, growth hormone supplementation, mitotane and trilostane.

Diagnosis of Typical Cushing's Syndrome

A presumptive diagnosis of typical or atypical hyperadrenocorticism can be made from clinical signs, physical examination, routine laboratory tests, and diagnostic imaging findings, but the diagnosis must be confirmed by hormonal assay. Screening tests are designed to diagnose hyperadrenocorticism, i.e., to determine if the disease is present or not. Tests that fit into this category are the corticotropin (ACTH) stimulation test, low-dose dexamethasone suppression test, and the urinary cortisol:creatinine ratio. None of these tests are perfect, and all are capable of giving false-negative and false-positive test results.

When a dog suffering from hyperadrenocorticism shows a negative result with one of the screening tests, this should not be surprising inasmuch as no test will have 100% sensitivity. In such dog, an alternative screening test should be used to help confirm the diagnosis if hyperadrenocorticism is still strongly suspected. It is extremely important to remember, however that, false-positive results are common in dogs suffering from non-adrenal disease. Because such false-positive test results occur for all of the commonly employed screening tests (ACTH stimulation, low-dose dexamethasone suppression, urinary cortisol: creatinine ratio), the definitive diagnosis of hyperadrenocorticism should never be made purely on the basis of results of one or more of these screening tests, especially in dogs without classic signs of hyperadrenocorticism or in dogs with known non-adrenal disease. If a dog has no clinical signs of Cushing's syndrome, we do not recommend treatment.

Corticotropin (ACTH) stimulation test

The ACTH stimulation test is commonly used as a screening test for hyperadrenocorticism in dogs. The basis for this test is that dogs with pituitary-dependent hyperadrenocorticism or cortisol-secreting adrenal tumors, because of their increased adrenocortical mass, have the capacity to secrete excessive amounts of cortisol. Advantages of this test include convenience, since it is a simple and quick test to perform. In addition, the ACTH stimulation test is the best screening test for distinguishing dogs with spontaneous from those with iatrogenic hyperadrenocorticism and provides valuable baseline information for

monitoring mitotane or trilostane treatment .

The preferred method for ACTH stimulation testing in dogs is to determine serum cortisol concentrations before and 1 hour after the IV or IM injection of cosyntropin (Cortrosyn, Amphastar Pharmaceuticals, Rancho Cucamonga, CA 91730), administered at a dosage of at least 5 microgram/kg. This 5-microgram/kg dosage will result in maximum stimulation of the adrenocortical reserve, the most important criteria for any ACTH stimulation protocol. Following reconstitution, the cosyntropin solution appears to be stable and bioactive for at least 4 weeks when refrigerated and for 6 months when frozen.

In normal dogs, administration of a supraphysiological dose of ACTH, either cosyntropin or ACTH gel, produces a rise in serum cortisol to values usually greater than 10 mg/dl (>300 nmol/L). In contrast, dogs with hyperadrenocorticism tend to have an exaggerated response to ACTH administration, with post-ACTH serum cortisol concentrations rising to greater than 20 mg/dl (>600 nmol/L). The ACTH stimulation test identifies over half of dogs with cortisol-secreting adrenocortical tumors and about 85% of dogs with pituitary-dependent hyperadrenocorticism.

One advantage of using the ACTH stimulation test is that it is the only screening test that can identify dogs with iatrogenic hyperadrenocorticism. Dogs receiving chronic glucocorticoid therapy can develop all of the clinical features of naturally occurring hyperadrenocorticism; this can develop with injectable, oral, topical or ophthalmologic steroid preparations. In dogs with clinical signs and routine laboratory findings consistent with Cushing's syndrome (especially if any history of exogenous steroid treatment), the finding of a low-normal baseline serum cortisol concentration with little to no response to ACTH stimulation is diagnostic for iatrogenic hyperadrenocorticism.

Low-dose dexamethasone suppression test

The low-dose dexamethasone suppression test is considered by many to be the test of choice for the diagnosis of hyperadrenocorticism in dogs. Normally, glucocorticoids (e.g., dexamethasone) feed back onto the pituitary gland, turning off or suppressing ACTH secretion. As circulating ACTH falls, cortisol secretion from the adrenal cortex is also diminished (i.e., suppressed). This test takes advantage of the fact that, in dogs with hyperadrenocorticism, the pituitary-adrenal axis controlling ACTH and cortisol secretion is abnormally resistant to suppression by dexamethasone. Dexamethasone is used for the low-dose dexamethasone suppression test not only because it is a potent glucocorticoid, but also because it does not cross-react with the standard cortisol assays, allowing one to use serum (plasma) or urine measurements as an endpoint.

Compared to the ACTH stimulation test, the low-dose dexamethasone suppression test is much more sensitive in confirming hyperadrenocorticism, since the results are diagnostic in the virtually all dogs with cortisol-secreting adrenal tumors and in 90 to 95% of dogs with pituitary-dependent hyperadrenocorticism. However, in contrast to the ACTH stimulation test, the low-dose dexamethasone suppression test is not helpful in the detection of iatrogenic hyperadrenocorticism. The test is also affected by more variables than the ACTH stimulation test, takes 8 hours to complete and does not provide pre-treatment information that may be used in monitoring the effects of mitotane or trilostane therapy.

Two similar protocols have been described for low-dose dexamethasone suppression testing in dogs, both of which yield similar patterns of cortisol suppression. To perform this test, serum or plasma cortisol concentrations are determined before, 4, and 8 hours after the

administration of the dexamethasone preparation. Either a solution of dexamethasone in polyethylene glycol (0.015 mg/kg, IV or IM) or dexamethasone sodium phosphate (0.01 mg/kg, IV) can be administered for the test with equivalent results. In general, the dexamethasone is best diluted in sterile saline to allow for the dog to be dosed accurately (e.g., dilute 2 mg (2,000 mg) of dexamethasone with 5 ml of saline to make a final concentration of 400 mg/ml) Interpretation of the results of a low-dose dexamethasone suppression test must be based on the laboratory's normal range for the dose and preparation of dexamethasone administered.

If the low dose of dexamethasone fails to adequately suppress circulating cortisol concentrations in a dog with compatible clinical signs, this is consistent with a diagnosis of hyperadrenocorticism. While basal and 8-hour post-dexamethasone samples are most important for interpretation of the test, one or more samples taken at intermediate times (eg, 4 hours) during the test period may also prove helpful. Approximately 30% of dogs with pituitary-dependent hyperadrenocorticism have serum cortisol suppression at 4 hours < 1 mg/dl or <30 nmol/l), with a rise in cortisol values by 8 hours after dexamethasone administration. This escape from suppression is diagnostic for pituitary-dependent hyperadrenocorticism, and further tests to determine the cause of hyperadrenocorticism are not necessary. Failure to show adequate suppression of serum or plasma cortisol at both 4 and 8 hours (i.e., >1 mg/dl or >30 nmol/l) is diagnostic for hyperadrenocorticism but can not aid in determining the cause of the hyperadrenocorticism.

The specificity of the low dose dexamethasone suppression test, however, can be low, especially when measured in a population of sick dogs. In fact, the specificity of the low-dose dexamethasone suppression test is considerable lower than that of the ACTH stimulation test. Because of the low specificity of the low-dose dexamethasone suppression test, diagnosis of hyperadrenocorticism should never be based on results of a low-dose dexamethasone suppression test alone, especially in a dog with nonadrenal disease. It is best to delay testing for hyperadrenocorticism until the dog has recovered from the concurrent illness.

Urinary cortisol:creatinine ratio

Calculation of a cortisol:creatinine ratio by use of cortisol and creatinine concentrations measured in a single urine sample is a simple and valuable screening test for hyperadrenocorticism in dogs. The main advantages of the urine cortisol:creatinine ratio over the low dose dexamethasone suppression and ACTH stimulation tests include the test's convenience and high sensitivity.

Cortisol and its metabolites are normally excreted into the urine, with urine cortisol excretion rising with increased adrenal secretion of the hormone. By measuring cortisol in morning urine, an integration of cortisol secretion over a period of about 8 hours is achieved, thereby adjusting for the wide and rapid fluctuations in circulating cortisol concentrations. Because creatinine excretion is relatively constant when kidney function is stable, dividing the urine cortisol by the creatinine concentration negates the effect of urine volume (and therefore the degree of urinary concentration) in interpreting the urine cortisol concentration. To this end, the urine cortisol:creatinine ratio is determined by dividing the urine cortisol concentration (in $\mu\text{mol/L}$) by the urine creatinine concentration (in $\mu\text{mol/L}$). In most laboratories, the reference range for the ratio is generally less than 15-20 (there are no units associated with the cortisol:creatinine ratio).

To perform this test, the owner is instructed to collect morning urine samples at the same time of day (e.g., 0700 to 0800 hours) on 2 to 3 consecutive days. On the preceding evening, the

dog should have its last walk at the identical times (e.g., 2300 hours). No special precautions are needed for the urine collection itself. However, after collection, each of the urine samples should be kept refrigerated until the owner is able to bring the specimens to the veterinary hospital. This home-collection protocol avoids the “stress” of a car ride or visit to the veterinary clinic for sample collection, both of which may cause slight elevations in the ratio in dogs without evidence of hyperadrenocorticism.

After submission of the dog’s morning urine samples to the laboratory for determination of cortisol and creatinine concentrations, the veterinarian should average the results of the 2 to 3 urine cortisol:creatinine ratios. The mean urine cortisol:creatinine ratio clearly differentiates between clinically normal dogs and dogs with hyperadrenocorticism (i.e, it is a sensitive diagnostic test). Unfortunately, the cortisol:creatinine ratio is also high (false-positive) in many dogs with non-adrenal illness. Therefore, while this simple test appears highly sensitive in detecting hyperadrenocorticism in dogs, it lack specificity and commonly produces false-positive results in dogs with severe nonadrenal illness.

Overall, because of the high sensitivity and low specificity of the urinary cortisol:creatinine ratio, it is recommended that this test be used primarily for its negative predictive value. In other words, if the results of cortisol:creatinine ratio remain within reference range limits, the presence of hyperadrenocorticism is highly unlikely. On the other hand, if a high cortisol:creatinine ratio is found, especially in a dog with concurrent disease (e.g., diabetes mellitus), we recommend this positive result for Cushing’s syndrome be confirmed with use of a low-dose dexamethasone suppression or ACTH stimulation test.

Diagnosis of Atypical and Pseudo-Cushing’s Syndrome

Although in most dogs with hyperadrenocorticism the diagnosis is straight forward, there are some dogs with clinical signs suggestive of hyperadrenocorticism that have normal or borderline cortisol testing on routine endocrine testing. Despite the fact that these dogs with overt clinical signs of hyperadrenocorticism lack evidence for cortisol excess by routine endocrine testing, many will still have a positive response to treatment for hyperadrenocorticism. These dogs have been described as having a syndrome termed “atypical“ hyperadrenocorticism. Measurement of serum concentrations of other adrenal steroid hormones, before and after ACTH stimulation, may assist in diagnosis of such cases.

Steroid Hormone Profiles after ACTH Stimulation

A number of laboratories offer individual adrenal steroid hormone assays. The Clinical Endocrinology Service at the University of Tennessee offers the most extensive adrenal steroid hormone profile. Their profile includes androstenedione, estradiol, progesterone, 17 hydroxyprogesterone, and aldosterone. The protocol for running the test is identical to that for a standard ACTH stimulation test, but the profile requires a larger sample (2.0 ml) of serum.

Most dogs with **classic hyperadrenocorticism** (confirmed by use of ACTH stimulation or low-dose dexamethasone suppression tests) also have elevations of other steroid adrenal hormones both before and after stimulation with ACTH. Hormones that are commonly increased include dehydroepiandrosterone, androstenedione, progesterone, and 17-hydroxyprogesterone. Although testosterone and estradiol concentrations are evaluated as part of some adrenal steroid hormone panels, they are less commonly abnormal. Profiles observed in dogs with hyperadrenocorticism vary from dog to dog, but most dogs with classical hyperadrenocorticism

have at least one steroid hormone (in addition to cortisol) that is increased. Typically two to three hormone concentrations are abnormal; however, it is unusual for all hormones in the profile to be increased.

Adrenal steroid hormone concentrations may be increased in dogs with either pituitary-dependent hyperadrenocorticism or adrenocortical tumor. Although the highest concentrations are typically found in dogs with adrenocortical carcinomas, there is extensive overlap between dogs with pituitary-dependent hyperadrenocorticism and adrenocortical tumor.

Concentration of 17-hydroxyprogesterone has been evaluated most extensively in dogs with hyperadrenocorticism. The percentage of dogs with hyperadrenocorticism that have an increase in 17-hydroxyprogesterone after stimulation with ACTH ranges from 55 to 85%. Thus, sensitivity of 17-hydroxyprogesterone for diagnosis of classical hyperadrenocorticism is lower than that reported for measurement of cortisol after ACTH stimulation.

Some dogs with **non-adrenal illness** also have increases in 17-hydroxyprogesterone secretions. In a group of dogs with non-adrenal neoplasia that did not have clinical signs of hyperadrenocorticism, a third had high post-ACTH 17-hydroxyprogesterone concentrations, whereas only 10% had high cortisol concentrations after ACTH stimulation. This suggests that the specificity of 17-hydroxyprogesterone for diagnosis of hyperadrenocorticism is lower than that of serum cortisol. Similar results were reported for measurement of corticosterone concentration.

In **dogs with atypical hyperadrenocorticism**, serum cortisol concentrations measured during routine endocrine function testing (with ACTH stimulation, low-dose dexamethasone suppression, or urine cortisol:creatinine ratio) are within or below the reference range, while other adrenal steroid hormone concentrations may be increased. The reason for lack of hypercortisolemia in dogs with atypical hyperadrenocorticism is poorly understood. In those with adrenocortical tumor, mutations within neoplastic adrenal tissue may lead to a blockade of cortisol synthesis (see section on non-cortisol secreting adrenal tumors), but atypical hyperadrenocorticism has also been well documented in dogs with pituitary-dependent hyperadrenocorticism. It is likely that in these dogs have increased adrenal steroid hormone concentrations due to adrenal gland hyperplasia and increased secretion of all adrenal gland products. It is possible that 24-hour production of cortisol is abnormal in these dogs, even though random circulating cortisol concentration is within reference range limits. Further studies are necessary to investigate the cause of atypical hyperadrenocorticism, especially in dogs with pituitary-dependent hyperadrenocorticism.

In dogs with clinical signs supportive of hyperadrenocorticism that have normal or borderline results on routine cortisol testing, marked increases in two or three adrenal steroid hormone concentrations are very supportive of a diagnosis of atypical hyperadrenocorticism.

In some dogs and cats with functional adrenocortical tumor, adrenal steroid hormones other than cortisol are the major secretory product of the tumor, and serum cortisol concentrations are low. Increased production of adrenal steroids other than cortisol may be due to deficiencies of one or more enzymes involved in normal steroidogenic pathways, possibly due to mutations in neoplastic adrenal tissue. Deficiency of these enzymes causes accumulation of precursor steroids proximal to the blocked step, with shunting of precursors into other metabolic pathways. Increases in enzyme activity may also play a role in steroid hypersecretion. Circulating cortisol concentrations in these dogs are hypothesized to be low because of suppression of the hypothalamic-pituitary axis by high circulating concentrations of progestin or other adrenal sex hormones.

All reported cases of non-cortisol secreting adrenal tumors in dogs and cats have been carcinomas. This is similar to the situation in humans in which adrenal carcinomas are usually inefficient in conversion of cholesterol to cortisol, and production of cortisol precursors is disproportionately high. In contrast, adrenal adenomas generally exhibit very efficient steroidogenesis and production of precursors is low or normal in relation to cortisol production.

In dogs and cats with non-cortisol secreting adrenocortical carcinomas, clinical signs are generally consistent with hyperadrenocorticism, and adrenal tumors are identified by imaging studies. However, endocrine function tests do not demonstrate hypercortisolemia, and results of ACTH stimulation testing typically show low cortisol concentrations that do not increase normally after ACTH administration. Hormones that have been reported to be increased in different combinations are 17-hydroxyprogesterone, progesterone, estradiol, testosterone, and androstenedione. Increased concentrations of corticosterone, and aldosterone have also been reported in dogs and cats with non-cortisol secreting adrenocortical carcinomas.

Pseudo-Cushing's (Alopecia X) is a form of canine adult-onset alopecia that may be due to mild hyperadrenocorticism. The problem affects Nordic breeds (Alaskan malamute, chow chow, keeshond, Pomeranian, Samoyed, Siberian Husky) and also the miniature poodle. Affected dogs have no other clinical signs of systemic illness; some studies have demonstrated borderline ACTH stimulation and low-dose dexamethasone suppression test results and mild increases in the cortisol:creatinine ratios in affected dogs. A mild form of hyperadrenocorticism is suspected in at least some affected dogs. Many dogs with alopecia X have increased serum sex hormone concentrations, both basally and following ACTH stimulation testing. The most frequent abnormalities detected include increased concentrations of progesterone, androstenedione, and 17-hydroxyprogesterone, although not all dogs have values outside the reference range.

Clinical Indications for Adrenal Steroid Hormone Panel

At this time, it is not recommended that adrenal steroid hormone measurement be utilized for routine diagnosis of hyperadrenocorticism. However, measurement of a panel of adrenal steroid hormones before and after ACTH stimulation should be considered in dogs that have clinical signs and clinical laboratory evidence for hyperadrenocorticism, no evidence of another cause for their clinical signs, and normal or borderline cortisol testing on routine endocrine testing. In a dog with clinical signs of hyperadrenocorticism, marked increases (1.5-2.0 times greater than the high end of the reference range) of at least two, and preferably three adrenal steroid hormone concentrations is consistent with a diagnosis of atypical hyperadrenocorticism.

Adrenal steroid hormone measurement should also be considered in dogs with clinical signs of hyperadrenocorticism and suppressed cortisol concentrations after ACTH stimulation after treatment with exogenous steroids or mitotane can be ruled out; this is particularly true if an adrenal mass is visualized with ultrasound or CT.

Measurement of an adrenal steroid hormone profile may also be useful in dogs with suspected alopecia X. Because of the paucity of information about the specificity of these measurements, every effort should be made to rule out other causes for the observed clinical signs prior to running an adrenal steroid hormone panel.

Treatment of Typical Cushing's Syndrome

The choice of treatment for a given dog with typical hyperadrenocorticism depends on

several factors including cause, severity of disease, presence of malignancy, available treatment options, and clinician and client preferences. For pituitary-dependent hyperadrenocorticism, bar far the most common cause of the syndrome, the 3 major choices are mitotane, trilostane.

Mitotane (Lysodren)

Mitotane (o,p'-DDD; Lysodren, Bristol-Myers Oncology Division) is the drug most frequently used for the treatment of dogs with pituitary-dependent hyperadrenocorticism. Mitotane is an adrenocorticolytic agent with a direct cytotoxic effect on the adrenal cortex, resulting in selective, progressive necrosis and atrophy. Since mitotane is a fat-soluble drug, its absorption is poor when administered orally to fasted dogs. Therefore, mitotane should be administered with meals.

Induction Dosage—The initial recommended mitotane dosage is 40 to 50 mg/kg/day PO generally administered for 7 to 10 days. It is very important that it be given with food to increase its absorption, and also to decrease the chances of it causing vomiting. Note that there appears to be no reliable method of predicting the length of time required for a response or the amount of mitotane therapy. However, it is unusual for a dog to require more than 10 consecutive days of mitotane. The owner should be advised to watch for a subtle change in appetite and water consumption. The mitotane should be stopped and an ACTH stimulation test done when any of the following occur: 1. the dog takes longer to consume any meal or certainly if it develops partial or complete inappetence.; 2. the dog vomits or has diarrhea; 3. the dog develops listlessness. If none of these develop, the ACTH stimulation test should always be repeated on day 10.

Monitoring Induction Dosage—The efficacy of the initial 7- to 10-day induction period is determined by an ACTH (Cortrosyn) stimulation test. Daily glucocorticoid administration must be withheld on the morning of ACTH stimulation testing, because prednisone and prednisolone both cross-react in most cortisol assays, resulting in a falsely high serum cortisol concentration. To ensure adequate control of hyperadrenocorticism, both the basal and post-ACTH serum cortisol concentrations must be lowered into the basal reference range (approximately 1-4 ug/dl or 25-125 nmol/L). The length of daily treatment needed to adequately reduce adrenal reserve varies and can range from 5 days to 2 months. The initial 7- to 10-day induction treatment is sufficient in most dogs.

In dogs still responding to exogenous ACTH with serum cortisol concentration above the basal reference range, daily mitotane administration should be continued and ACTH stimulation tests repeated at 7- to 10-day intervals until the serum cortisol concentration falls into the basal reference range. On the other hand, if low basal or post-ACTH cortisol concentration develops after the initial treatment period, mitotane should be withheld and glucocorticoid continued until the cortisol value rises to within the basal reference range. This usually takes 2 to 6 weeks but can take up to 12 to 18 months in rare instances.

Maintenance Dosage—Maintenance mitotane at a dosage of 50 mg/kg/week in 2 to 3 divided doses is instituted once daily treatment has successfully reduced adrenal reserve. While routine glucocorticoid supplementation is rarely necessary during maintenance mitotane treatment, an appropriate dosage of glucocorticoid should be administered during periods of stress.

Monitoring Maintenance Dosage—An ACTH stimulation test is performed after 1, 3, and 6 months of treatment and every 6 months thereafter to evaluate the efficacy of maintenance mitotane administration. If the basal or post-ACTH serum cortisol concentration increases above

the basal reference range, indicating a relapse, daily mitotane is reinstated at 40-50 mg/kg for at least 5 days; the weekly maintenance dosage is subsequently increased by approximately 50% after adrenal reserve has been appropriately reduced.

Relapses are common during maintenance mitotane administration, occurring in about half of dogs during the first year of treatment. Because of repeated relapse, maintenance dosages as high as 300 mg/kg/week may be necessary to control signs of hyperadrenocorticism in some dogs. Several factors may contribute to relapse in these dogs. One important factor under control of the clinician is the administration of an initial weekly maintenance dosage of at least 50 mg/kg. Less than 25% of dogs can be maintained long-term with less than 50 mg/kg/week, and very few with less than 40 mg/kg/week.

Adverse Effects—Side effects (ie, lethargy, weakness, anorexia, vomiting, diarrhea, and ataxia) are relatively common during mitotane administration, and clients should be advised of their occurrence. During the daily induction period, approximately one quarter of dogs develop one or more of these adverse reactions; however, they are relatively mild in most dogs and usually resolve quickly when mitotane is discontinued and glucocorticoid supplementation is increased. Similarly, if side effects develop during maintenance mitotane administration, the drug is discontinued and glucocorticoid supplementation given. If adverse clinical signs continue for longer than a few hours after cessation of mitotane and administration of glucocorticoid, the dog should be evaluated and an ACTH stimulation test be performed as soon as possible to exclude other disorders including mineralocorticoid insufficiency. If the adverse clinical signs were caused by a low circulating cortisol concentration, maintenance dosages of mitotane can usually be restarted in 2 to 4 weeks.

Complete glucocorticoid and mineralocorticoid insufficiency (ie, Addison's disease) develops in about 5% of dogs during maintenance mitotane administration, usually during the first year of treatment, although it can occur at anytime. It is not possible to predict which dogs will develop Addison's disease. Dogs developing iatrogenic Addison's disease almost always invariably glucocorticoid and mineralocorticoid replacement administration for the remainder of their lives, and additional mitotane administration is not required.

L-deprenyl (Anapryl)

L-deprenyl (selegiline hydrochloride, Anipryl, Deprenyl Animal Health, Inc., Kansas) is a monoamine oxidase inhibitor that inhibits ACTH secretion by increasing dopaminergic tone to the hypothalamic-pituitary axis. The use of l-deprenyl for treatment of hyperadrenocorticism has been evaluated in dogs. Although the effectiveness of treatment is relatively poor (10% response), one major advantage of L-deprenyl is the lack of any severe side effects, including iatrogenic hypoadrenocorticism.

Current recommendations—Treatment is initiated at a dosage of 1 mg/kg daily. If an inadequate response is seen after 1 month, the dosage is increased to 2 mg/kg/day for an additional month. If this dosage also proves ineffective, alternative treatment is necessary. If effective, daily treatment is continued for the remainder of the dog's life.

Monitoring Response—Response to treatment is evaluated by clinical signs and results of low-dose dexamethasone suppression test. Up to 90% of dogs may fail to adequately respond to treatment.

Contraindications—L-deprenyl appears to be a safe treatment alternative in most dogs with hyperadrenocorticism. However, L-deprenyl is not currently recommended for treatment of pituitary-dependent hyperadrenocorticism in dogs with concurrent diabetes mellitus, pancreatitis,

heart failure, renal disease, or other severe illness. In general, the use of the drug has become infrequent because of its poor response.

Trilostane (Vetoryl)

Trilostane is a synthetic steroid that competitively inhibits steroid synthesis by blocking 3 β -hydroxysteroid dehydrogenase. The adrenal glands and in particular the synthesis of glucocorticoids are more susceptible to its action than other steroid producing tissues. The reasons for this are not known. The Vetoryl brand of trilostane (Dechra Veterinary Products) was FDA-approved for use in dog in the United States late in 2008.

Vetoryl is available as 10, 30 and 60 mg capsules, and is readily available through most veterinary distributors.

Initial Dosage—Although few pharmacokinetic studies have been performed, trilostane is known to be short acting. The recommended starting dose is 2-3 mg/kg orally once daily, using the lower dose range in small dogs. Trilostane is absorbed better if given with food. It is effective in resolving the signs of pituitary-dependent hyperadrenocorticism in about 75% of cases. Polyuria, polydipsia, and polyphagia should dissipate within 4 weeks after starting trilostane. If this has not happened, then the dose should be increased. Skin changes should resolve within 4 months of starting treatment. All these improvements should be maintained as long as the dogs remain on adequate doses of trilostane.

Monitoring Trilostane Dosage—The efficacy of the drug and the required dosages are assessed using ACTH stimulation tests carried out 7 to 14 days, 30 days, and 90 days after starting therapy. ACTH stimulation tests should be started 4 hours after dosing; however, it is probably acceptable to start as early as 2 hours after dosing and as late as 5 hours (such that the last sample is then taken at 6 hours). There are significant differences between the cortisol responses to ACTH if stimulations tests are performed at other times. Many dogs require a change in dose (increase or decrease), and much higher doses may be required in some cases. Doses up to 40 to 50 mg/kg (divided twice daily) have been given with no unwanted side effects.

Post-ACTH cortisol concentrations should be between 40 nmol (1.5 μ g/dl) and 150 nmol/l (5.6 μ g/dl). If the post-ACTH cortisol concentration is lower, then trilostane is stopped for 5-7 days and then re-introduced at a lower dose. If the post-ACTH cortisol concentration is higher, then the dose of trilostane may need to be increased, depending on the resolution of clinical signs. If however the post-ACTH cortisol concentration is between these two values and the patient appears not to be clinically well controlled then the trilostane may need to be given twice daily. If an ACTH stimulation test is performed inadvertently at times other than 2 to 6 hours after dosing with trilostane, the post-ACTH cortisol concentration should be > 20 nmol/l (0.7 μ g/dl) and < 250 nmol/l (9 μ g/dl).

Once the clinical condition of the animal and the dose rate has been stabilized, the dog should be examined and an ACTH stimulation test performed every 3 months. Serum biochemistry (especially potassium measurement) can be performed periodically to check for hyperkalemia but with increasing clinical experience this becomes less important. Many dogs will show relapses at some stage and will require adjustments to the dosage of trilostane.

Adverse Effects—Minor side effects are sometimes seen, such as mild lethargy, decreased appetite and slight electrolyte abnormalities. These may occur from 2 to 4 days after the start of the therapy and are often transient and respond to dose reduction. If more serious signs of vomiting, diarrhea or lethargy develop, trilostane should be stopped and prednisolone

given for 1 or 2 days. In some cases dogs may require very much lower doses for the remainder of their lives. This has been linked to acute adrenal necrosis, the cause of which remains unknown.

Very few dogs develop signs of hypoadrenocorticism when treated with trilostane, although mild asymptomatic hyperkalemia is quite common. When hypoadrenocorticism does occur, dogs usually rapidly recover with appropriate therapy. A very few dogs have died despite withdrawal of trilostane and administration of appropriate therapy. A few sudden deaths without signs of hypoadrenocorticism while on trilostane therapy have also occurred; the role of trilostane in these cases has not been determined. The low prevalence of side effects compares favorably to those reported with mitotane.

Long-term adverse effects have not been documented, but it has been suggested that adrenal glands increase in size in response to therapy, probably as a result of chronic overstimulation with endogenous ACTH. There have been no documented instances of adrenal tumors developing in trilostane treated dogs.

Survival Time—The survival of dogs with pituitary-dependent hyperadrenocorticism treated with trilostane or mitotane was recently compared. There was no significant difference between the 123 trilostane treated dogs, surviving a median of 662 days (range 8-1971), and the 25 mitotane treated dogs, surviving a median of 708 days (range 33-1339). A comparison of twice-daily trilostane with mitotane induced adrenocorticolysis also did not demonstrate a significant difference.

It is important to note that many aspects of trilostane use in canine hyperadrenocorticism are still under investigation and, therefore, the above recommendations may change. Veterinarians who are unfamiliar with the use of the drug should consult the manufacturer or recognized specialist for up-to-date information.

Retinoic Acid

In human patients, many of the secretory pituitary tumors (eg, GH-secreting tumors and prolactinomas) can be controlled medically. However, very few human patients with Cushing's disease have been controlled medically, despite that fact that this disease (like in canine Cushing's disease) is almost always caused by an ACTH-secreting pituitary tumor. Recently, investigators have reported promising results with retinoic acid, the oxidized form of Vitamin A, in decreasing corticotroph secretion and proliferation in rodent models. Retinoic acid appears to inhibit ACTH through an action on POMC gene transcription and also inhibits pituitary ACTH-secreting adenoma development and proliferation.

Recently, Castillo and colleagues (2006) reported on the use of retinoic acid (2 mg/kg/day) in dogs with Cushing's disease for a period of 180 days. Clinical signs, plasma ACTH and alpha-MSH, the cortisol/creatinine urine ratio, and pituitary magnetic resonance imaging were assessed and compared at different time points.

In this study, there was a significant reduction in plasma ACTH and alpha-MSH, as well as in the cortisol/creatinine urine ratio, of the dogs treated with retinoic acid. Pituitary adenoma size was also significantly reduced at the end of retinoic acid treatment. Survival time and all the clinical signs evaluated showed an improvement in the retinoic-acid-treated dogs. No adverse events or signs of hepatotoxicity were observed, suggesting that the drug is not only effective but also safe. Overall, retinoic acid treatment controls ACTH and cortisol hyperactivity and tumor size in dogs with ACTH-secreting tumors, leading to resolution of the clinical signs of Cushing's disease.

Unfortunately, the cost of the retinoic acid, at least in the doses used for treatment of the dogs with Cushing's disease, in the study was *hundreds of dollars per week* for your average dog! Therefore, it is unlikely that this would be a very practical drug for most practicing veterinarians to use, at least at this point. More research is certainly needed to see if lower dosages would have any effect.

Cabergoline

The presence and inhibitory action of dopaminergic receptors in the anterior pituitary lobe is well known, and dopaminergic drugs such as bromocriptine and cabergoline, have long been used in dogs to inhibit the secretion of prolactin for reproductive problems. Cabergoline is a dopamine D2 agonist with a higher affinity and longer half-life than bromocriptine.

Dogs with Cushing's disease can have pituitary tumors that arise from either the pars distalis (anterior lobe) or pars intermedia (intermediate lobe) of the pituitary gland. In both cases, it is known that the secretion of ACTH and alpha-MSH can be inhibited by dopamine.

In another recent study by Castillo and colleagues (2007), 40 dogs with PDH that were treated with cabergoline (0.07mg/kg/week, divided into every other day treatments). The only side effect observed was vomiting, which occurred 1 hour after taking the first and/or second dose; no additional vomiting was observed despite continued administration. Out of the 40 dogs treated, 24 (60%) responded after the first month whereas 16 (40%) failed completely. Of the dogs that showed a favorable response, 7 of the 24 later became non-responsive with return of clinical signs of Cushing's disease. Therefore, a full prolonged response was observed in 17 (42.5%) of the 40 dogs, which were followed for up to 4 years.

A year after the treatment, there was a significant decrease in ACTH, alpha-MSH, urinary cortisol/creatinine ratio, and of the tumor size evaluated by nuclear magnetic resonance. Dogs responding to cabergoline lived significantly longer than those in the control group.

Overall, cabergoline appears to be useful treatment for dogs with Cushing's disease. Additional studies are justified, especially to see if combination treatment with mitotane or trilostane would improve the response.

Treatment of Atypical Hyperadrenocorticism

Dogs with pituitary-dependent *atypical* hyperadrenocorticism generally respond well to routine therapy for hyperadrenocorticism, as outlined above. In addition, however, since many of these dogs show only mild signs suggestive of Cushing's disease, use of drugs with less chance of toxicity may be sometimes recommended as initial treatment.

Mitotane and Trilostane

Good clinical responses have been reported with both mitotane and trilostane. No advantage of trilostane treatment over mitotane has been documented in dogs with atypical hyperadrenocorticism. In fact, some dogs with atypical Cushing's disease may have a better response to mitotane, inasmuch as trilostane treatment can elevate the serum concentrations of many of the sex hormones that appear to be involved with atypical Cushing's disease.

With both mitotane and trilostane, the drug doses should be monitored using the cortisol response to ACTH stimulation. Monitoring of the complete adrenal steroid hormone profiles during treatment is not currently recommended because of expense and limited usefulness. For example, in dogs with atypical hyperadrenocorticism successfully treated with mitotane, 17-

hydroxyprogesterone concentrations decrease; in those treated with trilostane, 17-hydroxyprogesterone concentrations increase, despite a good clinical response to therapy. Since trilostane inhibits the 3 β -dehydrogenase enzyme, 17-hydroxyprogesterone would be predicted to decrease, not increase. It is possible that trilostane either acts at additional steps in the synthetic cascade or that there is cross-reactivity of the assay for 17-hydroxyprogesterone with precursors such as pregnenolone and hydroxypregnenolone.

Melatonin

Melatonin can inhibit the activities of both 21-hydroxylase and aromatase enzymes within the adrenal cortex. Inhibition of the 21-hydroxylase enzymes could potentially lower serum cortisol concentrations, whereas inhibition of the aromatase enzyme could potentially lower circulating estradiol levels.

Therefore, melatonin treatment for cases of atypical adrenal disease in dogs may be effective, and particularly in cases where sex steroids (especially estrogens) are increased.

Flax Seed Oil plus Lignans

Flax seeds are among the highest known sources of lignans, chemicals found in plant that is a potent phytoestrogen. These phytoestrogens are estrogen-like chemicals and also act as antioxidants. (One must be careful NOT to confuse these *lignans* with *lignins*, a fiber like substance). Some *flax seed oils* in particular are advertised as being high in lignans.

The principal plant lignan precursor found in flaxseed is the phytoestrogen, secoisolariciresinol diglucoside (SDG). SDG is metabolized by bacteria in the colon to the mammalian lignans enterodiol and enterolactone, both of which possess weak and/or anti-estrogenic activities.

So again, like melatonin, the rationale of using flax seed oil that contains high amounts of lignans is that these drugs act as anti-estrogenic agents. In some dogs, these phytoestrogens help control signs of atypical Cushing's disease.

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