

Cushing's Syndrome with Concurrent Diabetes Mellitus in a Rhesus Monkey

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Abstract | Cushing's syndrome is the clinical expression of the overproduction of glucocorticoids and is well recognized in both human and veterinary medicine. Spontaneous diabetes mellitus is well known in *Macaca spp.*, however the occurrence of hyperadrenocorticism and diabetes mellitus concurrently in macaques has not been reported previously. This unusual case presents a rare opportunity to examine the relationships between two important endocrine diseases in a nonhuman primate. A male, 14-year-old rhesus macaque (*Macaca mulatta*) was diagnosed with hyperadrenocorticism and concurrent diabetes mellitus. Initially, the monkey had mildly elevated blood glucose values upon routine semi-annual physical examination. Further diagnostic work-up demonstrated hypercortisolism. Adrenocorticotrophic hormone-dependent Cushing's syndrome was subsequently diagnosed in light of results from dexamethasone testing, magnetic resonance imaging, and computed tomography scans. A therapeutic course of L-deprenyl (Anipryl®) was begun, and 8 weeks later, insulin therapy was initiated. The patient responded well to insulin therapy, however the dosage was rapidly increased. After 6 months, Anipryl® therapy was determined to be of little or no value, and ketoconazole was selected as the drug of choice to control the hypercortisolism. The monkey has shown remarkable improvement with the dual therapies of insulin and ketoconazole. Approximately 2 months after the initiation of ketoconazole therapy, the animal was returned to an experimental protocol under the conditions of twice-daily treatment and strict dietary control. The ongoing plan for clinical management includes periodic blood glucose and liver function surveillance.

Introduction

Cushing's syndrome is the clinical expression of the overproduction of glucocorticoid and is well recognized in both human and veterinary medicine. Diabetes mellitus also occurs in *Macaca spp.*, however diabetes mellitus secondary to hyperadrenocorticism has not been reported previously in macaques. There is a relative paucity of information dealing with hyperadrenocorticism of nonhuman primates, specifically macaques (1). The occurrence of two serious endocrine disease entities in the same monkey simultaneously is uncommon. The authors are aware of no other published descriptions of a clinical case of Cushing's syndrome with concurrent diabetes mellitus in a rhesus macaque. Much of the data accumulated from clinical cases is based on reports from humans and dogs. Moreover, there are no reports of experimentally induced animals models of both diseases concurrently. Recently, a diagnostic and therapeutic challenge was presented by a case of hypercortisolism and diabetes mellitus in a rhesus macaque. The present case offers a unique opportunity to study the interrelationship of these two serious diseases in a nonhuman primate.

Materials and Methods

A male, 14-year-old, rhesus macaque was housed individually in a 6.0 ft² stainless-steel cage in an AAALAC-accredited facility. All animal care and husbandry was conducted in accordance with *The Guide for the Care and Use of Laboratory Animals*. The room was environmentally controlled to provide a temperature between 18°C and 29°C, a relative humidity of 30%–70%, 100% fresh-air exchange at a rate of 10 to 15 per hour, and a light/dark cycle of 12:12. The monkey was fed a commercial monkey feed (Old World Primate Chow #5038; Purina Mills, St. Louis, MO) and Dustless Precision Pellets (Bio-Serv®; Frenchtown, NJ). Water was available *ad libitum* via an automatic watering system.

Routine physical examinations were conducted semiannually and included serum chemistry profile (Ektachem 250 Analyzer, Johnson & Johnson Clinical Diagnostics, Rochester, NY) and complete blood count (Baker System 9110, Biochem Immuno Systems, Allentown, PA). Tuberculin skin testing was done quarterly. The monkey tested serologically negative for *Cercopithecine* herpesvirus 1, SIV, STLTV, and SRV annually. Likewise, annual virus isolation tests for SRV were negative.

Established serum chemistry techniques at our Institute were used to monitor blood glucose concentrations (Ektachem 250). Low- and high-dose dexamethasone suppression tests (Dexamethasone, Phoenix Scientific, St. Joseph, MO) were used to differentiate the form of Cushing's syndrome. Plasma cortisol samples were assayed in duplicate by using reagents from Diagnostics Products Corporation, Los Angeles, CA (assay sensitivity was 0.2 µg/dl). Plasma samples were assayed for adrenocorticotrophic hormone (ACTH) in duplicate by immunoradiometric assay using reagents from Nichols Institute Diagnostics, San Juan Capistrano, CA (assay sensitivity was 2.0 pg/ml). Magnetic resonance imaging (MRI) was used to image the pituitary gland twice, with and without contrast media (GE Omega, 2 Tesla, 45-cm bore, 3 gau/cm; General Electric, Freemont, CA). The thorax and abdomen were scanned by computed tomography (CT; GE Hi Speed, General Electric Medical Systems, Milwaukee, WI). Medical therapies included insulin (Humulin® 70/30, Eli Lilly and Co., Indianapolis, IN), L-deprenyl (Anipryl®, Deprenyl Animal Health, Inc., Overland Park, KS), and ketoconazole (Nizoral®, Janssen Pharmaceutica, Titusville, NJ).

Case Report

Initially, during a routine physical examination, the monkey was noted to have a slightly elevated blood glucose concentration (190 mg/dl; normal, 50–100 mg/dl). The monkey was otherwise normal on physical examination and other serum chemistries. The mild elevation in glucose concentration was not investigated at the time. Approximately 5 months later, physical examination again indicated mildly increased blood glucose concentration (137 mg/dl). Glucosuria and ketouria were also

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noted at this time. Clinical signs of disease remained absent. In light of the patient's age (14 years) and lack of clinical signs, adult-onset non-insulin-dependent diabetes mellitus (type 2) was suspected. Dietary management, consisting of a nutritionally correct total biscuit count, multiple small feedings, and the cessation of food enrichment was implemented. Although the monkey had a good appetite and sufficient caloric intake to maintain ideal body weight, he began to lose weight. Blood glucose continued to increase (204 mg/dl) over the next 6 months despite dietary management. Serum biochemistry parameters were normal except for moderately elevated ALP values and slightly elevated GGT, ALT, and AST values. The hemogram was unremarkable.

The differential diagnosis included diabetes mellitus, hyperadrenocorticism, and liver disease. To rule out hyperadrenocorticism, plasma cortisol levels were measured. Elevated plasma cortisol and ACTH concentrations as compared to levels in a control monkey confirmed hypercortisolism. The control monkey was housed under identical conditions and assigned to the same protocol. The low-dose dexamethasone test suggested pituitary-dependent hyperadrenocorticism, and this diagnosis was supported by the results of both the plasma ACTH concentration and a high-dose dexamethasone test. An MRI of the brain was performed in an attempt to document the presence of a pituitary lesion responsible for the hypercortisolism. No pituitary lesion was found; however a pituitary problem could not be ruled out.

A novel approach to treatment was attempted by using L-deprenyl (Anipryl®) at a dosage rate of 2 mg orally daily. Anipryl® acts to correct dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis by increasing central dopamine levels, which in turn lower plasma ACTH and cortisol concentrations. At this time, the animal was below normal weight, with only minimal muscle mass and body fat. The hair coat was sparse, and the skin was thinner than normal but no striae or ecchymoses were present. The patient's mentation appeared dull, and he exhibited minimal physical activity.

After 10 weeks of Anipryl® therapy, blood glucose concentrations continued to increase. Plasma cortisol testing was repeated, and blood insulin concentration was measured. These parameters were compared to those of the same control animal as before. Laboratory results demonstrated a markedly elevated plasma cortisol concentration and a dramatically low serum insulin concentration ($< 1.0 \mu\text{U}$, control animal, $30.2 \mu\text{U}$). In conjunction with dietary management, insulin therapy was initiated at the dose of 2 units intramuscularly twice daily. Although Anipryl® therapy was thought to be of minimal value, therapy was continued to avoid confounding the clinical presentation.

Approximately 3 months after the first MRI was performed, a second MRI using gadolinium (Magnevist®, Berlex Laboratories, Wayne, NJ) (2) was performed. A pituitary lesion could not be identified. Plain radiographs of the thorax and abdomen were unremarkable. The insulin dose necessary to control hyperglycemia increased incrementally to a maximum of 15 units twice daily. The necessity for large amounts of insulin to achieve control over blood glucose was attributed to the hypercortisolism.

Nearly 6 weeks after the second MRI, a second course of dexamethasone testing, according to a modified version of the method developed by Liddle (3), was performed to document cortisol levels and objectively assess the efficacy of Anipryl®. As anticipated, these results supported earlier findings and indicated that Anipryl® therapy was not efficacious in reducing plasma cortisol. The clinical improvement, as evidenced by weight gain and mentation, was attributed to the insulin therapy. As expected, the hyperglycemia was poorly controlled, reflecting insulin resistance, and required increasing insulin dosages above 15 units

twice daily. Anipryl® was discontinued at this time, and an alternative sought. Because of its reversibility, low incidence of complications, rapid onset of action, efficacy, and ease of monitoring, ketoconazole was selected to control hypercortisolism.

Ketoconazole therapy was instituted at a dose of 50 mg/kg orally twice daily in a specially compounded pediatric elixir. The animal's clinical signs improved within 2 weeks of initiating the dual therapies of insulin and ketoconazole. Over the next 3 weeks, the animal gained more than 2 kg, his pelage regrew, and his mentation returned to normal. Liver function enzymes and plasma cortisol were monitored to adjust the ketoconazole dosage to the lowest effective dose. After 5 weeks of dual treatment, the animal was returned to the experimental protocol. The macaque continues to receive 22 units of insulin intramuscularly twice daily, 20 mg/kg of ketoconazole orally twice daily, and dietary management, which includes a gradual reintroduction of selected food-enrichment items. A dramatic change in the monkey's health is obvious and is supported by serum biochemistry results.

Roughly 6 weeks after the initiation of ketoconazole therapy, a CT scan was performed. The CT scan provided an opportunity to locate an ectopic source of the hypercortisolism and the possibility of a surgical cure. No lesions were found in the thorax or abdomen; however bilateral adrenal hyperplasia was documented. These findings support the likelihood of a pituitary microadenoma (Cushing's disease), too small for detection on MRI, causing over-stimulation of the adrenal glands and resulting in hypercortisolemia. The possibility, though remote, of an ectopic source of ACTH or cortisol releasing hormone (CRH) remains, as no lesion was found. After 2 months of ketoconazole therapy, the animal was returned to an experimental protocol under the conditions of twice-daily treatment and strict dietary control. The on-going plan for clinical management includes periodic blood glucose and liver surveillance.

Discussion

This patient first presented with hyperglycemia, therefore diabetes mellitus was placed at the top of the differential diagnoses. Testing regimens routinely used for the diagnosis of diabetes mellitus in human medicine, and to some extent in veterinary medicine, such as glucose tolerance tests were not conducted in this case because of the requirement for prolonged chemical restraint and the lack of established standards. There are reports in both human and veterinary literature of the use of glycated proteins to document hyperglycemia, however this technique is most useful as a monitoring aid to assess antecedent glycemic control (4, 5). Other rule-outs on the differential diagnosis list were hyperadrenocorticism and liver disease. Use of dexamethasone testing confirmed the diagnosis of Cushing's syndrome. Physicians rely on determination of 24-h urinary free-cortisol excretion, in addition to serum measurements. However, this technique was thought impractical in the present case (2).

The confirmation of Cushing's syndrome presented two new problems: first, the accuracy of the original diagnosis of diabetes mellitus and, second, the form of Cushing's syndrome present. As hypercortisolism provokes insulin resistance, it was reasoned that correction of hypercortisolism would ameliorate insulin resistance and the signs of diabetes mellitus. Anipryl® therapy was initiated to control hypercortisolism. Anipryl® is an irreversible monoamine oxidase type B (MOA-B) inhibitor that acts to restore depleted central dopamine levels and to facilitate dopaminergic transmission by a variety of mechanisms (6). The HPA axis is thought to be regulated in part by dopamine. For example, dogs with Cushing's disease show dysregulation of the HPA axis and dopamine depletion. The relative lack of dopamine then promotes an increase in the production and secretion of ACTH

and subsequently cortisol. Anipryl® corrects the HPA axis dysregulation by enhancing the dopamine concentration, which then down-regulates ACTH activity and normalizes plasma cortisol concentration (6). Anipryl® is used in humans in the treatment of Parkinson's disease because of its effects on dopamine metabolism. In addition, Anipryl® increases the synthesis and release of dopamine into the synaptic cleft and, by inhibiting MOA-B, retards dopamine re-uptake (7). The rationale behind the use of Anipryl® to treat canine Cushing's disease rests on the "hypothalamic" theory of the pathogenesis of pituitary adenomas. This theory is based on the role of altered hypothalamic control mechanisms that lead to tumorigenesis and hormonal overproduction. There is some evidence in dogs to suggest that HPA axis dysregulation is responsible for pituitary-dependent hyperadrenocorticism. Dopamine has been singled out as the most likely neurotransmitter involved (6, 8). A deficiency or blockade of dopamine causes an increase in ACTH secretion from the pituitary pars distalis (6, 9). It is unknown whether a similar mechanism applies to macaques, but Anipryl® proved unsuccessful in the case presented here.

Insulin therapy was initiated cautiously at low doses. It quickly became apparent that larger doses of insulin were required to control hyperglycemia. After we recognized the failure of Anipryl®, insulin was continued and ketoconazole substituted to control hypercortisolism. Ketoconazole is an imidazole derivative and potent antifungal agent. It is used as an antifungal agent at lower doses and inhibits steroid biosynthesis at higher dosages. Ketoconazole is most potent in suppressing androgen secretion (10) and, to a lesser extent, blocks cortisol biosynthesis (10, 11). Englehardt et al. found that ketoconazole was effective in almost all human patients with benign, primary Cushing's disease and that ketoconazole was beneficial for most patients with ectopic Cushing's syndrome; the drug improved biochemical and hormonal parameters, with few adverse effects (10). Feldman et al. evaluated ketoconazole in over 50 dogs with spontaneous hyperadrenocorticism due to Cushing's disease and adrenocortical tumors (9). Their experiences demonstrated that about 80% of animals had a rapid reduction in plasma cortisol concentration and cortisol responsiveness to ACTH. Dogs treated for more than 2 months demonstrated significant improvement of clinical signs. However some dogs failed to respond to treatment; this usually was due to poor intestinal absorption in most cases, but may also have been idiopathic. In light of these reports, we decided on a trial course of ketoconazole in the macaque. Indefinite, twice-daily treatment along with the expense of ketoconazole are two important considerations in the veterinary use of ketoconazole. The main side effect of ketoconazole is liver toxicity. However ketoconazole does have a low incidence of toxicity and negligible effects on mineralocorticoid production. Liver toxicity is easily monitored through periodic routine serum biochemistry screening (9).

Secondary diabetes mellitus occurs in humans, dogs, and cats that develop glucose intolerance secondary to concurrent insulin-resistant diseases such as hyperadrenocorticism. These cases usually begin with hyperinsulinemia, which is expected with insulin resistance. However, over time, pancreatic beta cell production dwindles, and permanent diabetes mellitus, usually insulin dependent, develops. This scenario seems to best fit the clinical findings in the presented case. However, the two disease entities may have arisen independently of one another. Because Cushing's syndrome was not diagnosed prior to the diagnosis of diabetes mellitus, we cannot conclude that the diabetes mellitus seen the macaque was a result of Cushing's syndrome. In light of the persistent hypoinsulinemia in this patient, this monkey likely will require exogenous insulin for the rest of its life, even if a definitive cure for the hyperadrenocorticism is found. In most cases, surgery is the treatment of choice for human

Cushing's disease patients, however locating and removing the source is necessary (12–14). Location of the causative lesion can be difficult or impossible, and sometimes the lesion is not operable.

Computed tomography scans have been useful in the diagnosis of tumors in man (2, 14, 15). Because the low risk and the marked potential benefit, CT was deemed worthwhile in the macaque. Inferior venous petrosal sampling and CRH testing are two sophisticated techniques used in human medicine to confirm the presence of pituitary lesions. It is possible to perform both of these techniques in a rhesus macaque, but due to the expense and the specialized facilities and equipment required, it is usually not practical, as in this case. In the search for ectopic sources of ACTH, techniques have been developed to sample venous blood at various sites along the inferior vena cava to monitor ACTH concentrations at the various sites (16). Samples having increased ACTH concentration over background levels direct attention to that organ or region of the body. This technique also was considered impractical in the presented case.

Two very striking features of the reported case are that the monkey was hypoinsulinemic, not normo- or hyperinsulinemic, and that the animal was not ketotic as a result. Typically hyperinsulinemia is a consequence of hyperadrenocorticism and its diabetogenic effects. This scenario may have occurred early in the progression of the disease, but seems to have disappeared as beta cell production of insulin declined. Ketoacidosis is a serious expected sequelae to insulin-dependent diabetes mellitus and fortunately did not occur in this patient. Ketoacidosis, and its very deleterious consequences, did not manifest probably because the minimal insulin production that remained was sufficient to prevent overt ketoacidosis. In some cases of diabetes mellitus secondary to hyperadrenocorticism, dependence on exogenous insulin declines as the insulin-resistance state resolves (9, 17). The extent of this recovery is thought to hinge upon the length of the insulin-resistant state and its damage to pancreatic beta cells.

The monkey in this case exhibited many of the clinical signs associated with hyperadrenocorticism in man and animals. The patient was subjectively polyuric and polydipsic, polyphagic, and thin-skinned with muscle atrophy and had poor haircoat (hairloss), reduced activity, and dulled behavior. The initial laboratory findings included mild hyperglycemia and a slightly elevated triglyceride level. Approximately 6 months later, serum biochemistry showed mild hyperglycemia with elevations in several liver enzymes (ALP, ALT, AST, GGT). Subsequent serum biochemistry panels demonstrated a continuation of this trend. Although cholesterol and triglycerides were elevated occasionally, these findings were not consistent. The RBC count indicated a downward trend, which began about 1 year after the original diagnosis and corrected shortly after ketoconazole therapy was initiated. Serum calcium was elevated during this same period. The expected hemogram alterations (elevated hemoglobin, PCV, and RBC count with lymphopenia and eosinopenia) were not seen in the case we describe and possibly reflect the relative lack of environmental changes in macro-environment, stressors, and microflora. The elevated liver enzymes are consistent the diagnosis of hyperadrenocorticism, but a variety of disorders may cause these elevations and thus are not specific to the diagnosis of hyperadrenocorticism. Measurement of the steroid-induced isoenzyme of ALP (SIALP) does not distinguish between common causes of hyperadrenocorticism, but its absence may allow the clinician to rule out hyperadrenocorticism (9).

Treatment of the diabetes mellitus in this case was straightforward, and the animal began to respond to insulin once the proper dose was reached. The treatment for hyperadrenocorticism was more challenging. Transphenoidal microsurgery to remove pituitary tumors is highly successful in human patients and offers

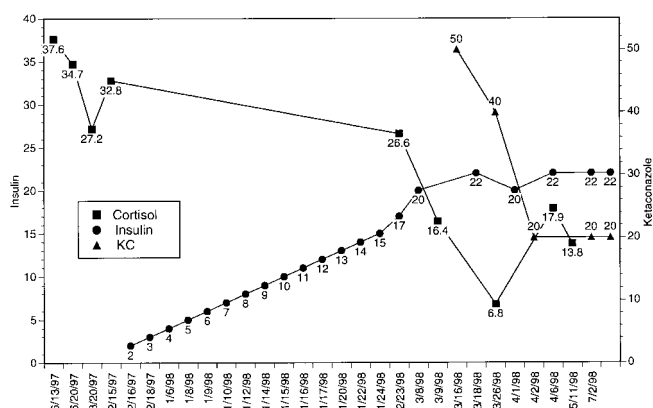


FIG. 1. Demonstrates the effect of ketoconazole on plasma cortisol. Note that the lowered plasma cortisol did not result in a decrement of the exogenous insulin requirement.

the best long-term outcome (2, 12, 13). There is an excellent description of a transoral, transphenoidal approach to the pituitary gland of the rhesus macaque by Tindall et al. The authors claim that the method is safe and practical for a variety of pituitary manipulations (18). The surgical option was not possible in this case because no pituitary lesion could be identified. Complete hypophysectomy was not attempted, mainly because of the serious post-operative sequelae and the intensive life-long treatment attendant to this procedure.

In addition to L-deprenyl and ketoconazole, there are a variety of other medical options available to the clinician in the management of hyperadrenocorticism. Chemotherapy with mitotane (o,p'DDD) was first suggested in 1973. This drug is used in humans and dogs, and it is the most common treatment for canine Cushing's disease (9). The drug is a derivative of the insecticide DDT and causes severe, progressive necrosis of the adrenal cortex, primarily the zona fasciculata and, to a lesser extent, the zona reticularis and zona glomerulosa. As such, its use results in a "medical adrenalectomy" (10). An ACTH response test that indicates hypoadrenocorticism is the goal of mitotane treatment in dogs. In humans, marked reduction in plasma cortisol concentration within 6 months is expected in 50% of patients (19). Mitotane was not selected as a therapeutic agent because it requires close monitoring, and aftercare can be intensive. Other drugs effective in the treatment of Cushing's syndrome include cyproheptadine, bromocriptine, metapyrone, aminoglutethimide, mifepristone, and etomidate (9, 10, 18).

In veterinary medicine, the medical management of Cushing's syndrome is primary, and surgery is seldom performed. Several diagnostic procedures were accomplished in this case that are not routine in veterinary practice, and surgery does remain an option for this monkey. The monkey described here continues to remain in good health with the dual therapies of insulin and ketoconazole. Blood glucose is monitored a minimum of twice weekly and often daily. The current insulin dose, although high, is required for control of hyperglycemia. As the hypercortisolism is corrected, the high insulin dose reflects the permanent damage to the beta cells and is not attributed solely to insulin resistance (Figure 1). Liver enzymes are monitored every 4 weeks. The monkey is observed at least twice daily during administration of treatments, so that changes in appetite or behavior are readily noted.

We intend to follow this animal's progress and continue to try to ascertain the origin of the hyperadrenocorticism. Because we were unable to definitively diagnose a pituitary lesion, the diagnosis in the macaque is best classified as Cushing's syndrome. Our therapeutic plan is based on ectopic ACTH syndrome. Because a surgical cure is not possible at this time, we have used

ketoconazole to block steroidogenesis. We intend to continue treatment and monitoring of the patient over the next year in the expectation that although a tumor has not been detected yet, it may become apparent during this time. At the end of the 12 months, the patient will be reevaluated with MRI and CT scanning as indicated (2, 13). If these efforts are unrewarding, we will continue medical therapy another year and reevaluate. Bilateral adrenalectomy is a last therapeutic resource but is unlikely to be performed due to complicated and intensive post-operative management.

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