

PHARMACOKINETICS OF PERGOLIDE IN NORMAL MARES

by

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Abstract

Objective: To determine the pharmacokinetic properties of oral pergolide in normal mares.

Animals: 6 horses, 3-17 years of age, 355-582 kg

Procedures: In a randomized, cross over design six healthy adult female horses received pergolide (PO) 0.01mg/kg or placebo after 8 hours of fasting. Samples were taken over a period of 6 day for each portion of the study (treatment or placebo) with a two week minimum wash out period between study periods. Quantification of pergolide concentration was determined by UPLC-MS. Quantification of α -MSH was determined by radioimmunoassay validated for horses. Quantification of ACTH concentration was determined by chemiluminescent enzyme immunoassay.

Results: Pergolide was detected in all treated horses. The relatively short time to peak concentration (0.5 hours) indicates a rapid absorption. Mean maximum concentration measured was $4.05 \text{ ng/ml} \pm 2.02$ with a median time to maximum concentration being 0.415 hours (range:0.33-1.0). The mean half life of pergolide was determined to be $5.86 \text{ hours} \pm 3.42$. Lower limits of quantitation for the UPLC-MS assay was 0.5 ng/ml. α -MSH results were evaluated using a multiple analysis of variance assay for repeated measures comparing treatment, time, and period. There was a significant treatment to period effect with $p=0.02$. The effect of period appears to be more significant ($p=0.06$) compared to the effect of treatment ($p=0.77$). No effect from pergolide was noted on ACTH concentrations.

Conclusions and Clinical Relevance: Horses appear to absorb and eliminate pergolide more rapidly than previously expected. Based on this pharmacokinetic data the dosing strategies of pergolide may need to be altered. However, assay sensitivity does need to be improved prior to recommendations being made.

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List of Abbreviations

Abbreviations for Figure 1.2: POMC=pro-opiomelanocortin,
ACTH=adrenocorticotropin

Abbreviations for Figure 1.3: POMC=pro-opiomelanocortin,
ACTH=adrenocorticotropin, α -MSH=alpha melanocyte stimulating hormone,
CLIP=corticotrophin-like intermediate peptide, β -END=beta-endorphin

Abbreviations for Table 2.1: LOQ=lower limits of quantification

Abbreviations for Table 2.2: K_{el} = slope of terminal portion, $T_{1/2}$ = half life, C_{max} = maximum concentration, AUC_{0-last} = area under the curve from 0 to the last measured concentration, $AUC_{0-\infty}$ = area under the curve from 0 to infinity, AUC % extrap= the % of the area under from 0 to infinity extrapolated from the last time point, MRT= mean amount of time a drug molecule spends in the body, T_{max} = time at which maximum concentration is reached, Apparent V_d/F = apparent volume of distribution, Apparent Cl/F = apparent clearance

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CHAPTER 1 - Literature Review

Over the last several decades, the number of geriatric horses presented to referral institutions has increased dramatically.^{7,36} This is likely a reflection of an increase in the number of older horses in the general population (estimate to be 7 to 20% of the horse population), and differences in attitudes in horse owners about viewing their horse as companion animals.^{7,36}

Pituitary pars intermedia dysfunction (PPID) or equine Cushing's disease is probably the most common disease of older horses.³⁸ There are at least 20 peer reviewed reports of PPID in horses published in the veterinary literature over the last decade. Clinical signs of PPID vary in severity and include hirsutism, hyperhidrosis, polyuria, polydipsia, loss of muscle tone, and abnormal fat distribution.³⁸ Progression of the disease can result in laminitis and/or immunosuppression that may require euthanasia. Horses with PPID have hypertrophy and hyperplasia of the pars intermedia which leads to over expression of proopiomelanocortin (POMC).^{35,38} There are several diagnostic tests for documenting PPID in horses.^{12,13,38} The most common include endogenous ACTH concentrations and an overnight dexamethasone suppression test.^{12,13} The treatment of choice for PPID is pergolide, a dopamine agonist. Pergolide was initially used in human medicine to treat Parkinson's disease and restless leg syndrome; however it has been pulled from the market due to cardiac complications. Pergolide has been used in horses for years to treat PPID; however there are no reports of its pharmacokinetic or pharmacodynamic properties. Treatment regimes of horses with PPID have typically been based on human literature and response to therapy.

Pituitary Gland

Anatomy

The pituitary gland, also called the hypophysis, is a small gland located ventral to the optic chiasm that connects to the hypothalamus via the pituitary stalk.¹⁶ Embryological development of the pituitary gland is from both neural and epithelial substrates.²³ Based on the embryologic tissue, the pituitary gland is divided into the neurohypophysis (neural tissue) and

the adenohypophysis (epithelial tissue).²³ The neurohypophysis is further divided into the pars nervosa and pars eminens.²³ The adenohypophysis is further divided into the pars tuberalis, pars intermedia, and pars anterior or pars distalis (Figure 1.1).^{23,38} Use of the terms anterior or posterior are anatomically incorrect for quadrupedal species, as the neurohypophysis usually lies dorsal to the remainder of the pituitary gland. The neurohypophysis is actually embedded within the adenohypophysis in the horse. The pars intermedia lies in a continuous rim located between the pars distalis and the pars nervosa.²³ Vascular supply to the neurohypophysis and pars intermedia is through both the portal veins (ventral and dorsal) as well as direct arterial supply from systemic circulation.³⁸ The pars distalis vascular supply is through the dorsal and ventral portal veins.²³

The neurohypophysis is made up of a collection of nerve axons and terminals that originate in the supraoptic and paraventricular nuclei of the hypothalamus.²⁶ The cell bodies of these neurons produce oxytocin and arginine vasopressin (or anti-diuretic hormone ADH) which is stored in the pars nervosa for release into systemic circulation.²⁶ The pars distalis is composed of a heterogeneous cell population with 5 different cell types. These include somatotrophs, lactotrophs, corticotrophs, gonadotrophs, and thyrotrophs.³⁸ The pars intermedia is composed of a single endocrine cell type, melanotrophs.

Function

The function of the adenohypophysis is to produce and store hormones necessary for daily physiologic functions.³⁸ Somatotroph cells produce growth hormone while lactotrophs produce prolactin.³⁸ Gonadotrophs produce hormones important for follicular growth and ovulation including follicle-stimulating hormone and luteinizing hormone. Thyrotrophs produce thyrotropin. These hormones are secreted based on either neural or hormonal stimulation from the hypothalamus.¹⁶ Most of the stimulation for the pars distalis comes from hypothalamic releasing factors delivered by the ventral and dorsal portal veins.²³ The exact function of the pars tuberalis is currently unknown.

Corticotrophs of the pars distalis and melanotrophs of the pars intermedia produce the same precursor protein pro-opiomelanocortin (POMC). Due to different posttranslational processing each cell type secretes a different complement of POMC-derived peptides. Corticotrophs primarily produce adrenocorticotropin (ACTH), while melanotrophs produce α -

melanocyte stimulating hormone (α -MSH), β -endorphin (β -END), corticotrophin-like intermediate peptide (CLIP), and only a very small amount of ACTH. In corticotrophs of the pars distalis, prohormone convertase I is present which converts the majority of the POMC protein into ACTH, see Figure 1.2.²⁶ In melanotrophs of the pars intermedia, prohormone convertase I and II are present. The presence of both hormones results in cleavage of the POMC protein into α -MSH, β -END, CLIP, and a small amount of ACTH, see Figure 1.3.²⁶ Further processing of the hormones produced is performed to regulate their activity and is affected by disease states, see Figure 1.4.²⁶

Melanotrophs of the pars intermedia are innervated by hypothalamic periventricular dopaminergic neurons.²⁶ The release of the neurotransmitter dopamine from these neurons causes tonic inhibition of release of hormones from surrounding melanotrophs.²⁶ After release dopamine binds to dopamine (D2) receptors on melanotrophs which inhibit the transcription of POMC and inhibit the release of POMC derived peptides.²⁶ Dopamine also inhibits cell proliferation of the melanotrophs in the pars intermedia. Melanotrophs are stimulated by the release of melanotrope releasing factor from the hypothalamus.²⁶ Exogenous thyroid releasing hormone (TRH) is known to stimulate melanotrophs secretory activity, however the physiological significance of this is unknown at this time.

The corticotrophs of pars distalis produce ACTH in response to pulsatile stimulation of corticotrophin releasing hormone (CRH) and arginine vasopressin released from the hypothalamus.³⁸ Once released ACTH acts on the zona fasciculata of the adrenal cortex which causes the production and release of cortisol (glucocorticoid). Inhibitory feedback on the release of ACTH occurs at the level of the hypothalamus and pars distalis in response to appropriate or high levels of adrenocortical steroids.²³ This inhibition is primarily mediated through type II receptors located in the arginine vasopressin and CRH secreting neurons as well as the corticotrophs of the pars distalis.²³ Although the pars intermedia does produce smaller amounts of ACTH, it does not experience the same inhibitory feedback as the pars distalis.²¹

The physiologic effects of the POMC related peptides including α -MSH, β -END and CLIP are not well documented in the horse, but are better described in other species.²⁶ α -MSH is primarily thought of as a hormone responsible for skin or coat pigmentation when it interacts with melanocortin receptor 1 which is found predominately on the skin.²⁶ Interaction with melanocortin receptor 3 and 4, which are found in the central nervous system especially the

hypothalamus, are essential for the control of energy homeostasis.²⁶ At this level their function is involved in the leptin-melanocortin pathway which regulates fat metabolism and appetite-satiety balance.²⁶ Obesity is found to affect humans and horses that lack these receptors. α -MSH has also been found to have profound anti-inflammatory effects with the regulation of the cytokine response by inhibiting the activation of nuclear factor- κ B by lipopolysaccharide and interferon- γ .²⁶ Recently McFarlane *et al.* in 2004 found that there is a significant rise in α -MSH in normal horses and ponies in the late summer and fall, which may correlate to decreasing daylight hours.²⁸ It is thought that this rise helps to prepare the horses for winter time and decreased food provisions. β -END has been found to provide analgesia (endogenous opioid), behavior modification, immune system modulation, and an effect on vascular tone.²⁶ CLIP has been studied the least of the POMC peptides but it is thought that it may have an effect on insulin secretion.²⁶

The POMC peptide, ACTH is primarily produced by the pars distalis. The primary physiological effects of ACTH include increased cortisol production. The physiological effects of cortisol are widespread, and include changes in metabolism of glucose, protein and fat. Cortisol stimulates gluconeogenesis through mobilization of extrahepatic amino acids and through the production of amino acids from carbohydrates in the liver.¹⁶ Cortisol also results in decreased glucose utilization by peripheral cells, which may be in part due to a decrease in insulin sensitivity. Glucocorticoids increases lipolysis for the production of fatty acids and further production of glucose.¹⁶ Cortisol also possesses several anti-inflammatory properties, which are also considered immunosuppressive. These effects are caused by multiple mechanisms which include the stabilization of lysosomal membranes, decreasing the migration of white blood cells into inflamed area, decreasing phagocytosis of damaged cells by white blood cells, decreasing permeability of capillaries, reducing the release and possibly binding of interleukin-1 from white blood cells, and decreasing T lymphocyte production or survival.¹⁶

Pituitary Pars Intermedia Dysfunction (PPID)

Pathophysiology

Although PPID has occurred in the geriatric horse population since first being recognized in 1932, the exact pathophysiology of the disease was not elucidated until the last 25 years.³⁸ It

was not until Millington *et al.* in 1988 found that affected horses had a decrease in dopamine concentrations in pituitary tissues that lead to the true understanding of the pathophysiology of this disease.³⁴ In this study Millington found that horses affected with PPID had an 88% reduction in dopamine concentrations compared to age matched controls.³⁴ Following this study McFarlane *et al.* in 2005 utilized immunohistochemistry to find that PPID affected horses had a 50% reduction in the number of periventricular cell bodies compared to age matched control horses.^{25,29} The data from these studies strongly support the theory that PPID is due to a dopaminergic neurodegeneration. It is noteworthy that PPID is a misnomer given that this syndrome is primarily a hypothalamic disease, and not a primary pituitary disease

Dopaminergic neurodegeneration in PPID is very similar to the pathology that occurs in Parkinson's disease, although there are significant differences. The primary clinical signs of Parkinson's disease are progressive motor dysfunction, while the primary clinical signs of PPID are due to hormonal dysfunction. The disparity of clinical signs is likely due to differences of the location of the dopaminergic neurodegeneration within the central nervous system. The motor dysfunction in Parkinson's disease is due to loss of nigrostriatal neurons, while the hormonal dysfunction in PPID is a result of loss of periventricular hypothalamic neurons.²⁵ The exact cause of the dopaminergic neurodegeneration is still being evaluated; however there is recent evidence that oxidative stress likely plays a role with both diseases. Reactive oxygen species produced during transmitter metabolism by the dopaminergic neurons are capable of damaging these neurons since they are exquisitely sensitive to their effect.²⁹ McFarlane *et al.* 2005 documented that aging alone results in an increase in oxidative stress. Aged horses have an increase amount of 3-nitrotyrosine and α -synuclein within the pars intermedia when compared to younger horses.²⁹ However, PPID horses had significantly higher amounts of 3-nitrotyrosine and α -synuclein even when compared to aged matched controls. The cause of the oxidative stress in normal geriatric horses and PPID horses is not a result of a reduction in systemic antioxidant capacity.³¹ However, this particular study suggested that there may be a decrease in the local antioxidant capacity within the pars intermedia of affected horses.

Dopamine, as discussed previously, is released from the hypothalamic periventricular neurons. It inhibits growth of the cells of the pars intermedia and decreases production of POMC peptide related hormones. With the loss of dopamine, the cells of the pars intermedia are allowed to become hypertrophic and hyperplastic. This proliferation of the pars intermedia cells

allows can result in macroadenomas (>1 cm in diameter) or microadenoma formation. Post-mortem examination will often reveal a grossly enlarged pituitary gland. When evaluating PPID affected horses Heinrichs *et al.* found that out of 19 horses, 13 horses had pars intermedia macroadenomas while only 6 had pars intermedia microadenomas.¹⁷ A majority of the macroadenomas observed replaced the pars distalis in affected horses. This finding was also appreciated by van der Kolk in 1996, when he evaluated 24 PPID affected horses that had pituitary glands that weight two to three times more than normal pituitary glands⁴³. The pars intermedia tumors typically have a low mitotic index and do not metastasize.³⁸ The tumors are also well vascularized and commonly have associated hemorrhages; however they do typically lack a distinct capsule.³⁸ Other tumors including pars distalis and a functional adrenocortical neoplasm have been associated with PPID like syndrome, however they are rare and do not have the same pathophysiology as tumors of the pars intermedia.^{6,45}

The loss of the inhibitory control of dopamine not only allows the cells of the pars intermedia to proliferate. These cells also produce and release increased levels of the POMC protein derivatives.³⁸ It is important to note that in other species serotonin plays a major role in production of POMC peptides, however the importance of serotonin in the pars intermedia of horses remains unclear.⁴¹ Immunostaining of affected pars intermedia has revealed an increase in the amount of α -MSH, β -MSH, γ -MSH, β -END, CLIP, and to a lesser extent ACTH. Normally the production and secretion of ACTH is under inhibitory feedback in the pars distalis. The pars intermedia does not respond to this inhibitory feedback, however production of ACTH in the pars distalis does diminish considerably.^{21,38,42} Although the majority of clinical signs associated with PPID are considered to be due to an increase in ACTH production, immunostaining has not provided support for this theory. Instead, it has been found that the MSH-related peptides and β -END, which increase dramatically, greatly potentiate the adrenosteriodogenic effects of ACTH.²¹

Clinical Signs

PPID is most commonly seen in horses over the age of 15 years, so it is considered a disease of the increasing geriatric population of horses. Overall, the incidence of PPID does not appear to be rising but our population of geriatric horses has increased as has our recognition of

the disease. PPID affects all genders. PPID affects all breeds, but in some studies ponies and Morgans were overrepresented.²⁵ There is evidence that ponies have a longer lifespan than horses. A table of number of horses, ages, and gender in 6 studies of PPID are listed in Table 1.1. Common clinical signs vary widely, but the most common clinical sign appreciated is hirsutism. Common clinical signs and their incidence in 6 studies are listed in Table 1.2.

Hirsutism is a unique clinical sign with horses with PPID and is described as an abnormally long curly coat that fails to shed. More subtle hair coat abnormalities will often develop before hirsutism. These changes include small areas of long hairs amongst a normal coat or a winter coat that comes in early and reluctant to leave the following spring.^{24,41} This mild abnormality may then proceed to a full thick coat of curly hair that does not shed normally. The coat may change to a lighter color in darker horses.^{38,42} The exact mechanism for the development of hirsutism in horses affected by PPID is not understood, however the current hypothesis is that it is due to chronic elevations of MSH peptides.³⁸ Another clinical sign of PPID that is not well understood is hyperhidrosis. A physical pressure caused by the enlarged pars intermedia on the thermoregulatory center of the hypothalamus, a direct effect of increased β -endorphin, or simply an inability to dissipate heat normally due to the long hair coat are possible proposed theories for the develop hyperhidrosis.^{24,38}

Polyuria and polydipsia (PU/PD) has also been reported in at least one third of horses affected with PPID.³⁸ The hypothesized mechanism of the cause of PU/PD in PPID horses varies greatly. Hypotheses primarily consider the overproduction of ACTH and cortisol the main causes, however this does not always appear to be the case. Hypothesis for PU/PD include: 1) hyperglycemia and glucosuria resulting in osmotic diuresis; 2) cortisol increases the glomerular filtration rate; 3) cortisol inhibits release and/or action of arginine vasopressin; 4) compression of the pars nervosa resulting in a decrease in arginine vasopressin, and 5) cortisol and ACTH inhibiting the renin-angiotensin-aldosterone axis.^{24,26,38,41} It has also been hypothesized that due to the immunosuppression activity of cortisol, a urinary tract infection may be present and cause PU/PD.^{24,38} PU/PD has also been associated with hyperhidrosis that commonly occurs in PPID affected horses. The true frequency of osmotic diuresis resulting in PU/PD is unknown, as glucosuria in horses with PPID and PU/PD was an uncommon finding in one report.³⁸

Abnormal fat distribution is commonly seen in horses with PPID. Common areas of fat deposition in PPID horses include the tailbase, supraorbital fossa, crest of the neck and

sheath.^{26,38} The proposed mechanism of this abnormal deposition of fat is due to hyperinsulinemia. However, as mentioned earlier the combination of the other more commonly produced POMC peptides may potentiate more of the signs commonly associated with increased cortisol production. Abnormal fat deposition is typically followed later in the disease process by weight loss and muscle wasting. Weight loss is most likely due to a combination of multiple problems including poor dentition, parasitism, chronic infections, reduced exercise, poor nutrition, and increased protein catabolism.²⁶ Muscle wasting appreciated in these horses is considered to be due to increase cortisol production. Muscle biopsies performed on affected horses reveal type 2 myofiber atrophy, which is consistent with corticosteroid muscle atrophy in other species.²⁶ The muscles most commonly affected are those muscles associated with the rump and the epaxial musculature.^{26,38} With this type of muscle loss and abnormal fat distribution, horses commonly develop a very round abdomen.³⁸

Chronic infections or delayed wound healing is another common finding in PPID affected horses. Common infections include parasitism, sinusitis, respiratory infections, skin infections, tooth root abscesses, pneumonia (broncho, pleuro, fungal), recurrent subsolar abscesses, and conjunctivitis.^{26,38,42} Many of these infections may initially respond to appropriate therapy and then either slow down recovery or have recurrent bouts of infection. PPID horses that develop broncho or pleuropneumonia typically have a mixed population of causative agents and can also have fungal involvement.^{38,42} Affected horses that are not on appropriate parasitic prophylaxis can develop severe intestinal parasitism.²⁶ Immunosuppression associated with PPID is commonly considered to be due to increased ACTH and thus cortisol production. However, as mentioned previously the other POMC peptides help to potentiate the effects of cortisol. Also, β -END itself has immunosuppressive effects, especially by decreasing T-cell blastogenesis. To evaluate immune system status in PPID affected horses, McFarlane *et al.* in 2008 evaluated cytokine distribution in aged horses, age-matched horses affected with PPID, and adult (non-geriatric) horses.³⁰ In this study, normal aged horses had increased concentrations of inflammatory cytokines, (including interleukin-6, interleukin-8, and interferon- γ); indicating that aging is associated with a pro-inflammatory state.³⁰ In contrast to the normal aged horses PPID affected horses had decreased levels of interleukin-6 and interferon- γ , but had very similar levels of interleukin-8.³⁰

Chronic laminitis occurs in PPID affected horses and has the potential to be a life-threatening complication. Chronic laminitis is reported to occur in 20 to 84% of horses affected by PPID.¹¹ Donaldson *et al.* in 2008 evaluated 40 horses with laminitis for evidence of PPID. In this study 70% of the horses appeared to be affected by PPID and horses appeared to be more likely to develop laminitis during September and May.¹¹ The exact pathophysiology in the development of laminitis still remains poorly understood and appears to be multifactorial. Most hypotheses revolve around the increase in ACTH/cortisol production or potentiation of its effects including hyperglycemia, insulin resistance, hyperinsulinemia, and the catabolic effect of cortisol. One hypothesis is that the insulin resistance induced by glucocorticoids starves the sensitive laminae tissue of glucose, its energy substrate, resulting in laminae death.²⁶ However, in a recent report GLUT-4 (an insulin dependent glucose receptor) was not detected in laminar tissue, and glucose uptake in lamellar tissues was independent of insulin.¹ Another hypothesis is that based on the catabolic nature of cortisol that the integrity of the laminae is diminished, leading to recurrent bouts of laminitis.¹¹ High concentrations of insulin may play a direct role in the development of laminitis. An experimental model resulting in hyperinsulinemia (and normoglycemia) resulted in laminitis in normal ponies.²

There are many other clinical signs associated with PPID, however these appear to be less common than the previously discussed clinical signs. Lethargy or a behavior change to a more subdued horse is considered to be due to an increase in β -END.⁴¹ This increase in β -END has also been considered to be the reason that some PPID affected horses have a decreased response to pain.⁴¹ Neurologic signs including seizures, abnormal vision, blindness, ataxia, and narcolepsy have been reported and may be associated with compression from a macroadenoma.^{38,41} Infertility and persistent lactation has also been reported in PPID affected mares.³⁸ The cause of persistent lactation is most likely due to the lack of dopaminergic inhibition of prolactin. Possible causes of infertility in these mares could be due to destruction of gonadotrophs by the pars intermedia adenoma or suppression of gonadotropin secretion by glucocorticoids.^{24,38} Another source of infertility could be the decreased ability to clear uterine infections. Despite reports of infertility in PPID affected horses there are reports of successful pregnancies and parturition occurring in PPID affected horses.³⁸

Another clinical sign more commonly seen in humans with hypercortisolism is osteoporosis.^{38,42} Specific reports of osteoporosis in horses with PPID are lacking. However

there are multiple incidences of PPID horses being euthanized after fractures or fractures during surgical recovery in PPID horses.³⁸ The exact pathologic process is not completely understood. Hypertrophic osteopathy has been reported in a pony with a pituitary adenoma.³⁸ Another musculoskeletal condition commonly associated with PPID is the breakdown of the suspensory apparatus especially hind limb, which can result in the necessity of euthanasia.

There are several abnormal clinicopathologic findings associated with PPID. Complete blood count (CBC) abnormalities reveal a stress leukogram consisting of a mature neutrophilia and an absolute lymphopenia.³⁸ Eosinopenia and anemia have also been described.^{38,41} Serum chemistry abnormalities include hyperglycemia, hypertriglyceridemia/hyperlipemia, increase in liver enzymes, and hypercholesterolemia. Glucosuria is reported in PPID affected horses in association with hyperglycemia.⁴¹ The majority of these abnormalities are hypothesized to occur due to increased levels of cortisol or the potentiated effect by the POMC peptides. Horses are typically resistant to hepatic changes induced by glucocorticoids, however with insulin resistance and increased lipolysis by the glucocorticoids fat disposition in the liver may occur.³⁸ Resting insulin concentrations are often increased, consistent with insulin resistance in horses with PPID.

Diagnosis

Multiple diagnostic tests exist for PPID, each with its own limitation. As we have used these tests over the years and have learned more about the disease itself, it has become clear that no test is perfect and will correctly diagnose each case. Validation for any diagnostic test requires comparison to a known gold standard. Postmortem histologic evaluation of the pars intermedia has been considered the gold standard for diagnosis of PPID. However, there is lack of consensus in histologic interpretation of pituitary glands from aged horses with mild clinical signs of PPID.³¹ Histologic changes of the pars intermedia of healthy aged horses likely overlap with lesions observed in early disease. Van der Kolk *et al.* in 2004 reported that pathological changes of the pituitary gland (cysts, hyperplasia, microadenomas, and macroadenomas) increases with age.⁴⁴ Additionally, this study also found that pregnancy and lactation resulted in an increase in pituitary weights. Season (likely due to decreasing daylight hours) also adversely affects diagnostic tests for PPID. POMC peptides concentrations (including ACTH and α -MSH) start to increase in the late summer and fall in normal horses. It is unknown if season

affects POMC peptide production in PPID horses. It is very likely that season affects all diagnostic tests for PPID.

There are numerous diagnostic tests that have been performed and evaluated over the years for PPID. Ante-mortem diagnostic tests include baseline plasma cortisol or loss of diurnal rhythm, dexamethasone suppression test, ACTH stimulation test, TRH stimulation test, combined TRH dexamethasone suppression test, baseline POMC peptide concentrations, serum insulin concentration, urinary corticoid-to-creatinine ratio, salivary cortisol concentration, domperidone ACTH stimulation test, and diagnostic imaging (radiographs, computed tomography, and magnetic resonance imaging).³⁸ Although each of these diagnostic tests has their own advantages and disadvantages it appears that none are without fault. Baseline plasma cortisol or loss of diurnal rhythm, dexamethasone suppression test, ACTH stimulation test, TRH stimulation test, combined TRH dexamethasone suppression test, endogenous POMC peptides, and domperidone ACTH stimulation test will be discussed individually as they have shown to be the diagnostic tests most commonly used and shown most promise in eliminating errors in testing.

Resting or Baseline Plasma Cortisol Concentration

This diagnostic test measures baseline cortisol in a horse without stimulation by ACTH or inhibitory feedback with glucocorticoids. This test is very difficult to interpret due to multiple causes such as that affected horses typically have baseline cortisol within reference range approximately 47% of the time.⁴² There is also the concern of the cortisol level being altered by stress or exercise prior to sample collection. Horses have been documented to have a diurnal rhythm with the most significant rise in cortisol in the morning; this can also affect test results depending on the time of sample collection.^{14,38} A suggested variation of this diagnostic test is the evaluation for a loss of the normal diurnal rhythm of the horse; this is considered to be less than 30% variance from the morning to evening sample of plasma cortisol.³⁸ This test has not been substantially validated in horses to be considered useful in the diagnosis of PPID, unless the use of glucocorticoids is prohibited.³⁸

Dexamethasone Suppression Test

This test has been considered the “gold standard” of the endocrine tests by many practitioners and researchers. There are several variations of the dexamethasone suppression test, including an overnight and 24 hour suppression test. The 24 hour dexamethasone suppression tests requires multiple samples, at inconvenient times, which makes this test less preferred than the overnight dexamethasone suppression test. Dexamethasone suppression tests rely on negative inhibitory feedback caused by dexamethasone on ACTH and cortisol secretion. Since the pars intermedia does produce ACTH but does not respond to the negative feedback of increased glucocorticoid, horses affected with PPID should not have suppressed cortisol concentrations after dexamethasone administration.^{24,26} Studies have shown that PPID affected horses do initially have cortisol suppression following dexamethasone, but that they break out of that suppression much sooner than normal horses.^{24,41} A dexamethasone suppression test is not without risk in PPID affected horses, especially those that have had laminitis. The administration of dexamethasone has the potential to exacerbate laminitic episodes in these horses.^{11,38}

The overnight dexamethasone suppression test requires collection of blood during one to three time points, which varies among reports.^{26,41} Blood is usually collected (for measurement of cortisol concentrations) immediately prior to administration of dexamethasone; however collection of this blood sample is not necessary. A dose of 40µg/kg body weight of dexamethasone is given intramuscularly at 5:00pm. Blood samples to evaluate for suppression of cortisol can be performed at 15 hours, however generally 19 hours post dexamethasone administration is considered to be adequate.^{26,41} Suppression is considered to have occurred when circulating cortisol is less than 1 µg/dl at the 19 hour sample. A diagnosis of PPID can be made if cortisol concentrations are greater than 1 µg/dl 19 hours dexamethasone administration.

The overnight dexamethasone suppression test was first reported by Dybdal *et al.* 1994, with 100% sensitivity and specificity when blood samples were collected 19 hours after dexamethasone administration.¹³ However, upon further clinical evaluation, the sensitivity and specificity of this test is probably much lower. There are several reasons that might explain the disparity between the initial report, and this observation. Sensitivity is likely affected by severity of the disease. Horses with mild PPID will likely have more cortisol suppression than horses with advanced disease. Season affects dexamethasone suppression tests results in normal horses, and false positive tests are more likely in the fall. Donaldson *et al.* in 2005 found that

cortisol concentrations were highest in September after performing an overnight dexamethasone suppression test.¹²

Adrenocorticotropin Stimulation Test

As previously mentioned the increase in circulating cortisol in PPID affected horses is due to pars intermedia adenoma formation; however in most humans and canines excess cortisol is due to an overactive adrenal cortex. Adrenal gland hyperplasia is reported in approximately 20% of PPID horses.³⁸ This test provides exogenous adrenocorticotropin (ACTH) and then evaluates cortisol levels as a response. This test is performed by administering ACTH intramuscularly (1 IU/kg ACTH gel) or intravenously (25 IU ACTH₁₋₂₄ or 100 IU of synthetic ACTH) then collecting blood samples to evaluate cortisol response. The intravenous testing method appears to be more beneficial with statistical significance identified between normal and PPID horses.⁴¹ This test is thought to be more beneficial in testing for adrenal gland function, especially when considering exhaustion. However, this test failed to discriminate between horses with PPID and normal horses, and is not recommended for testing for PPID.⁴¹

Thyrotropin-Releasing Hormone Stimulation Test

The thyrotropin-releasing hormone (TRH) stimulation test has been used for a number of years to evaluate horses with PPID. This test was first evaluated by Beech and Garcia where they administered 1mg TRH IV to healthy and PPID affected horses, then evaluated plasma cortisol concentrations.^{4,41} They found that thyroid hormones rose in all horses, as expected, however the cortisol levels in PPID horses rose significantly compared to control horses. In addition to evaluating TRH's effect on cortisol, Beech *et al.* in 2007 evaluated the effect of TRH on ACTH concentrations and compared it to the overnight dexamethasone suppression test in PPID and normal horses.³ In this study there was an increase in ACTH concentrations associated with TRH administration in all horses, with a significant increase in horses with PPID. It was determined from this study that the TRH stimulation test had a high sensitivity (86-100%) and specificity (24-88%), although the specificity was less than the overnight dexamethasone suppression test.³

The exact mechanism of how TRH causes an increase in cortisol has not been known until recently. McFarlane *et al.* in 2006 evaluated α -MSH concentrations in response to TRH administration. They found that plasma α -MSH concentrations increased by 600% in response

to TRH administration in all horses (normal and PPID horses).^{26,27} This response indicates that TRH stimulates the melanotrophs of the pars intermedia, which would then lead to increased levels of ACTH and cortisol.^{26,27}

The TRH stimulation test is performed by administering 1mg of TRH intravenously. Blood should be collected prior to administration; however there are multiple collection times listed in the literature but mainly include 15, 30, and 90 minutes post TRH administration.³⁸ The test is typically considered positive if plasma cortisol concentrations show a 30% increase between 15 and 90 minutes post-administration.³⁸ The main advantage of this test is that it does not involve the administration of dexamethasone which could result in laminitis in some PPID horses. Another advantage of this test is the short testing time period. Disadvantages of this test include the multiple sampling times and in the past TRH have been difficult to obtain. The improved availability of compounded TRH has decreased cost and difficulty in performing the test. Another concern about this test is the lack of an established sensitivity and specificity.²⁶

Combined Dexamethasone Suppression Test/Thyrotropin Releasing Hormone Stimulation Test

The combined dexamethasone suppression test TRH stimulation test was developed to decrease the influence of a variable initial cortisol concentration when using the TRH test alone.³⁸ This test is considered to be more sensitive since it does utilize two useful diagnostic tests in series. The conceptual explanation of this test is that by administering dexamethasone, cortisol concentration decreases. With the reduction in the baseline cortisol concentration, it is easier to discern the horse's response to TRH. The test is performed by administering 40µg/kg dexamethasone intravenously 3 hours prior to the administration of TRH (1 mg intravenously).^{38,41} Circulating cortisol levels are measured prior to TRH administration, then at 15 minutes, 30 minutes, 45 minutes, 60 minutes, 90 minutes, and 21 hours after TRH administration.⁴¹

This test was critically evaluated by Frank *et al.* in 2006, and test results were compared to the gold standard (histology).¹⁵ The combined test had a sensitivity of 88%, a specificity of 76%, a positive predictive value of 71%, and a negative predictive value of 90%, for the population studied (prevalence of PPID = 40%).¹⁵ In this study the results of the combination test was compared to the overnight dexamethasone suppression test alone, the TRH stimulation test alone, and histopathology.¹⁵ Based on their findings, it was determined that this test was more

sensitive than either component alone; however it was not as specific as the TRH stimulation test alone.¹⁵ Despite the advantage of improving the sensitivity of testing, this test is not used widely due to the amount of time required for administration and multiple blood samples that are required. Also, there is an increased cost to administer this test (time, samples, and drugs) and still increased risk for laminitis with the administration of dexamethasone.

Endogenous POMC peptides

The measurement of POMC peptides is considered to be the easiest, safest, and most cost effective test to diagnose PPID. ACTH is the most common peptide tested for and is available commercially. α -MSH and β -END have also been reported to be elevated in PPID horses. The sensitivity of measuring baseline ACTH has been reported to be 80% while the specificity has been reported to be 90%.²⁶ This estimate is likely over estimated since it is compared to the overnight dexamethasone suppression test as the gold standard.

Measuring ACTH concentration is used commonly because it eliminates multiple sample times as well as eliminating the administration of dexamethasone, thus providing a safer test for laminitis prone horses. It is important to note that even though ACTH concentrations may be elevated, circulating cortisol may be within normal limits. It is also important to remember that, as Donaldson *et al.* found, ACTH levels are significantly increased in September compared to January or May in even normal horses.¹² Testing at an inappropriate time of year could lead to false positives. A disadvantage of measuring ACTH concentrations is that it is a sensitive peptide that can be damaged by handling mistakes. In contrast α -MSH is less sensitive to handling mistakes and has been shown to be a possible diagnostic tool for PPID, although it is also affected by season.

Domperidone ACTH Stimulation Test

The domperidone ACTH stimulation test is a very recent test reported by Miller *et al.* in 2008.³³ The conceptual theory of this test is that since PPID results in pars intermedia hyperplasia, administration of domperidone (a dopamine antagonist) will result in an increase release of ACTH in PPID horses. Miller *et al.* evaluated not only the horses ACTH concentration but also pituitary histopathology assigning a grading system.³³ The test was performed by measuring plasma ACTH, then administering 3.3mg/kg domperidone orally. ACTH concentrations were measured 4 and 8 hours after domperidone administration.

Miller *et al.* assigned grades 1 through 5, with 1 being normal and 5 being a macroadenoma.³³ In this study they found that horses with histologic grades greater than or equal to 3 consistently had greater levels of circulating ACTH concentrations in response to domperidone administration.³³ Benefits of this test include that it is more sensitive than resting endogenous ACTH concentrations and that it does not involve the possible risk of laminitis associated with dexamethasone administration. Disadvantages of this test include multiple testing times and the cost associated with domperidone. The effect of season has not been critically evaluated with this test, however it would be assumed that as with other tests that measure ACTH it would be preferable to not test in the autumn of the year.

Treatment

Treatment of PPID in horses involves management changes as well as pharmacological therapy. Management changes in these horses are necessary not only because of their disease status but also due to the increased age that PPID typically occurs in horses. Pharmacological therapy for PPID horses has typically focused on replacing POMC inhibitory compounds, but has also included drugs that inhibit adrenal steroidogenesis.

Management changes for horses affected with PPID include body clipping to help horses affected with hirsutism and hyperhidrosis.³⁸ It is also imperative to provide adequate dental care to these horses to allow them to masticate normally and decrease the likelihood of tooth related problems that may be enhanced by the immunosuppressive nature of PPID. A proper diet that not only provides adequate nutrition but is also easily masticated is also beneficial for these horses. These horses should be monitored for weight loss and muscle loss over time, with appropriate adjustments made in feeding routine, dental care, and anthelmintics to correct for these problems. Since PPID horses are commonly affected with laminitis, attention to good hoof care is essential. Diet management and exercise can also help with decreasing the likelihood of development of laminitis. By decreasing non-structural carbohydrates and increasing insulin sensitivity with exercise, it appears that these horses are less susceptible to laminitis.³⁸ Infections incurred by PPID affected horses may need to be treated for longer periods of time with appropriate antibiotic therapy.

Pharmacologic therapy has focused on the decreased production of POMC peptides or decreasing the production cortisol from the adrenal cortex. Cyproheptadine, a nonselective 5-hydroxytryptamine receptor blocking agent (serotonin antagonist), was used initially due to the large effect it exerts on rat pituitary POMC peptide production.^{38,41} In humans, administration of cyproheptadine resulted in a decrease in ACTH and β -END secretion from ACTH-producing tumors.⁴¹ Cyproheptadine is given at 0.6 to 1.2 mg/kg orally every 24 hours, however other sources indicate that the appropriate dose is 0.3 to 0.5 mg/kg orally once daily.^{26,38} Adverse effects associated with cyproheptadine administration is rare even with double dose administration.³⁸

Several clinical studies have been performed to evaluate the effect cyproheptadine on PPID horses. These studies evaluated improvement of clinical signs and diagnostic test results. In a very small study, cyproheptadine improved clinical signs in only 1 out of 4 ponies affected with PPID.²² However, other studies by Couëtil and Perkins *et al.* found that cyproheptadine improved clinical signs and appeared to improve endocrine test results.^{8,37,39} Couëtil found that ACTH concentrations decreased, but rarely to normal values, while Perkins *et al.* found that ACTH concentrations did return to normal levels after treatment.^{8,37} Schott *et al.* found that follow up endocrinologic tests (dexamethasone suppression test, TRH stimulation test, insulin concentrations, and ACTH concentrations) did not show significant improvement in horses treated with cyproheptadine compared to untreated horses.³⁹ Results from these some of the studies could be confounded by changes made in management of these horses, since these were mainly field studies.

Dopamine agonists replace the inhibitory peptide dopamine to decrease production of POMC peptides. Orth *et al.* in 1982 found that an intravenous infusion of dopamine caused a significant decrease in POMC peptide concentrations.³⁵ Utilizing the theory that replacement of dopamine would suppress POMC peptide production, pergolide and bromocriptine have both been evaluated for the treatment of PPID in horses. Bromocriptine has been administered orally, intravenously, or by subcutaneous injection at a dose of 0.03 to 0.09 mg/kg every 12 hours.^{38,41} It has been shown to be an effective treatment of PPID; however it is not readily available commercially and appears to have a low bioavailability in the horses.³⁸ For these reasons, pergolide is the most common dopamine agonist used to treat PPID in horses.

There are several reports describing the effects of pergolide on clinical signs and on endocrine tests. Improvements in clinical signs are based on owner perceptions in most reports, and there are few well designed studies. Schott *et al.* reported clinical signs improved in over 80% of pergolide treated horses.³⁹ However, after endocrine evaluation of these same horses only about one third of these horses were normal.³⁹ Donaldson *et al.* in 2002 found that pergolide treated horses had a significant reduction in laminitis or laminitic episodes.¹⁰ In this same study, PPID horses had a significant decrease in plasma ACTH concentrations after initiation of pergolide treatment, in comparison to cyproheptadine treatment.¹⁰ However, there was not a untreated control group in this particular study. Orth *et al.* in 1982 evaluated the effect of oral pergolide (0.01 mg/kg) on POMC peptides at 0.01mg/kg in a single horse with PPID.³⁵ In this study there was a significant decline in ACTH, β -END, and α -MSH, although they did not reach normal levels.³⁵ He also noted that despite a decrease in ACTH, plasma cortisol only declined slightly for a short period of time.³⁵

Pergolide is given orally with a dose range of 0.006 to 0.01 mg/kg every 24 hours.³⁸ Improvement in clinical signs typically takes up to 6 weeks to occur; if improvements in clinical signs or endocrinologic tests are not appreciated the dose of pergolide used can be slowly increased. Adverse effects with pergolide treatment are rare but range from colic, anorexia, and diarrhea; these signs are typically associated with higher dose administration.³⁸ The use of pergolide has been enhanced by the increase in price of cyproheptadine, the availability of a less expensive compounded product, and the scientific literature confirming the beneficial effects of treatment with pergolide. Pergolide was recently pulled from human pharmacies due to complications with human Parkinson's patients, thus the only way to obtain pergolide is through compounding pharmacies. Davis *et al.* in 2009 evaluated the effects of compounding and storage condition on the stability of compounded pergolide.⁹ They found that once compounded into an aqueous vehicle, pergolide is unstable and should be kept protected from light, refrigerated, and not used 30 days after compounding.⁹

Drugs that inhibit adrenal steroidogenesis that have been used to treat PPID include trilostane, a competitive 3- β hydroxysteroid dehydrogenase, or o,p'-DDD, an adrenocorticolytic agent. O,p'-DDD acts to destroy the adrenal cortex that results in decreased cortisol secretion, while trilostane blocks cortisol synthesis by the adrenal cortex. Early studies reported poor results with the administration of o,p'-DDD.³⁸ However, a recent study performed by McGowan

and Neiger found a significant improvement in clinical signs and cortisol response to TRH stimulation test in PPID horses treated with trilostane.³² Twenty horses in this study received 0.4 to 1mg/kg orally once daily for 30 days without adverse effects noted.³² PPID was not confirmed with histopathology after treatment in these horses. It has been proposed that possibly multiple drugs should be used to treat PPID in horses, possibly a combination of pergolide and trilostane. It would be beneficial to detect adrenal hyperplasia prior to starting trilostane therapy in these horses, however this multilevel approach may be what is necessary for some previous unresponsive horses. It is unclear at this time if the combination of these drugs would have enough beneficial effects in PPID horses to warrant use, however this may be on the horizon for future studies.

Pergolide: Pharmacokinetic-Pharmacodynamic Modeling

Pharmacokinetics

Pharmacokinetic parameters for pergolide have only been evaluated in humans to this point. It was previously used to treat Parkinson disease and restless leg syndrome in humans until cardiac complications warranted it be pulled from the human market. Pergolide is a dopamine agonist acting mainly at D2 and D3 dopamine receptors, see chemical structure in Figure 1.5.⁴⁰ However, even in humans, the pharmacokinetics of pergolide have not been well documented, due to the small doses used, and the extensive first-pass metabolism resulting in very low plasma concentrations.^{5,20} Recent developments in the field of high-performance liquid chromatography (HPLC)-tandem mass spectrometry assay has allowed measurement of very low pergolide concentrations in plasma (lower limit of quantification: 10 pg ml⁻¹).^{5,19,20,40} In humans, pergolide is often administered at a dose of 1 mg q 8 hours (0.014 mg/kg based on a 70 kg body weight) for treatment of Parkinson's disease.⁴⁰ Peak plasma concentrations of the drug are achieved within 2-3 hours following oral administration.^{5,40} Pergolide has a large apparent volume of distribution (14,000 L) with a half-life of approximately 21 hours.^{5,40} The very large apparent volume of distribution is at least partially responsible for the low plasma concentrations.

Pharmacodynamics

The pharmacodynamics of pergolide in relation to pharmacokinetic evaluation has only been performed in humans. In human studies the evaluation of pharmacodynamics has typically focused on the patient's Unified Parkinson's Disease Rating Scale (UPDRS) motor score in relation to pergolide exposure.⁴⁰ In a study by Thalamas *et al.*, there was a significant correlation between decreased motor scores (or improved motor function) and the area under the plasma concentration-time curve (AUC).⁴⁰ There was a general improvement in motor function with increasing time to maximum concentration (C_{\max}), but this was not statistically significant

.⁴⁰

Figures and Tables

Figure 1.1: Equine Pituitary Gland

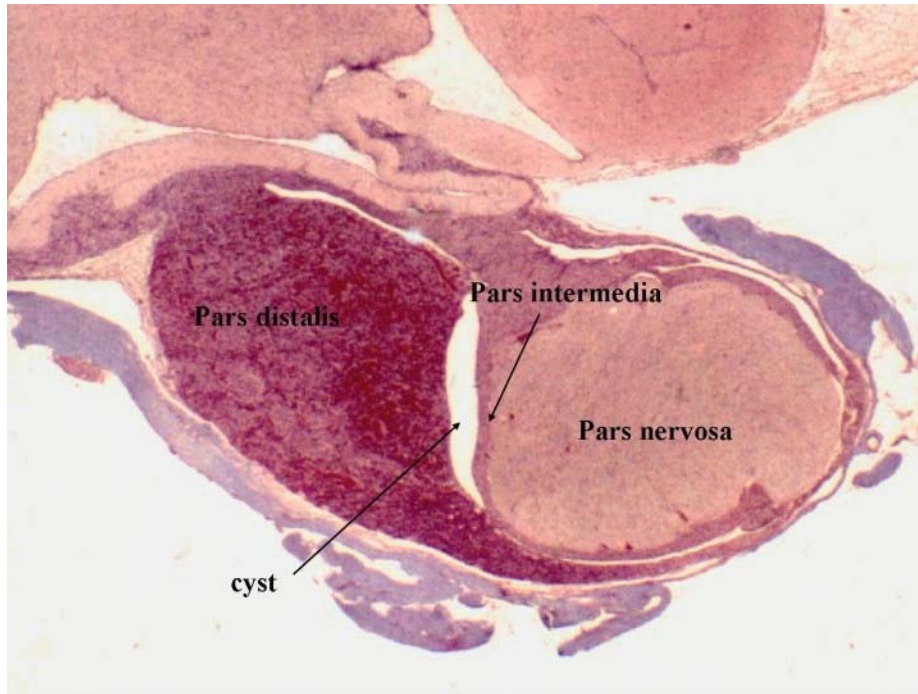


Figure 1.2: Pituitary POMC peptide metabolism in pars distalis

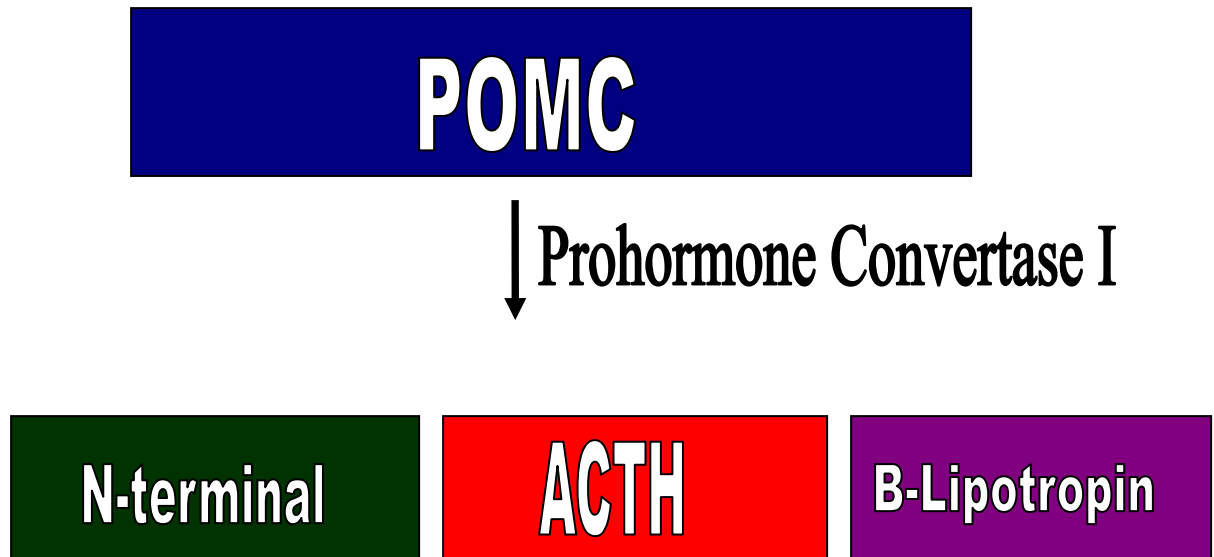


Figure 1.3: Pituitary POMC peptide metabolism in pars intermedia

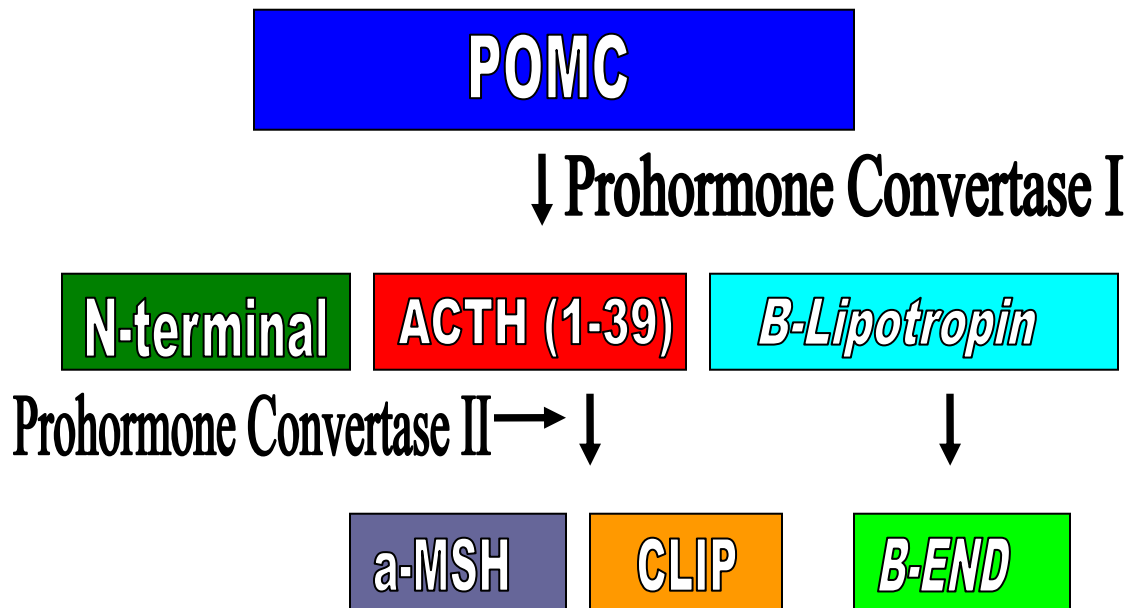


Figure 1.4: POMC pathway with pars intermedia or pars distalis/anterior processing, adapted from Schott et al.³⁸

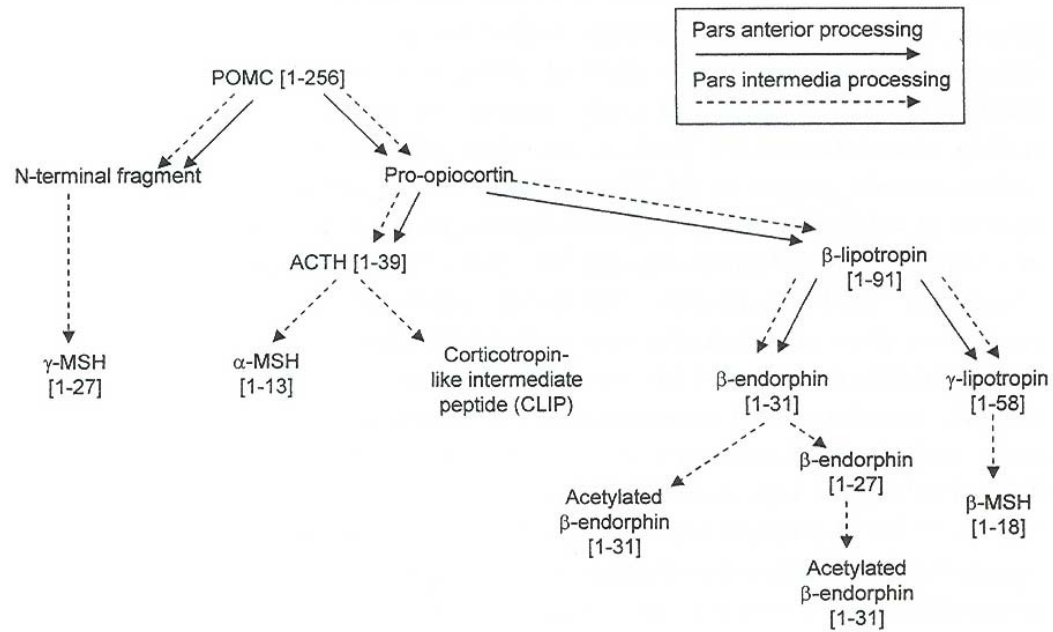


Figure 1.5: Pergolide chemical structure

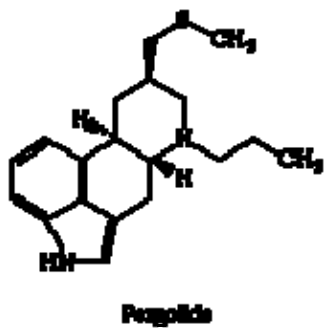


Table 1.1: Overview of number of PPID horses and ponies in current literature³⁸

	Heinrichs et al (1990) ¹⁷	Hillyer et al (1992) ¹⁸	Boujon et al (2003) ⁶	Van der Kolk et al (1993) ⁴⁶	Couetil et al (1996) ⁸	Schott et al (2001) ³⁹
Number of equids	19	17	5	21	22	77
Number of ponies	NR	11 (65%)	NR	12 (63%)	11 (50%)	15 (19%)
Age:Mean (range)	19.1 (7-31)	20.2 (12-34)	18.2 (13-24)	21 (12-30)	21.5 (8-31)	22.8 (12-34)
Sex	11♀/6♂/2NR	6♀/11♂	4♀/1♂	8♀/13♂	11♀/11♂	37♀/40♂

Table 1.2: Overview of clinical signs (%) seen in PPID horses from current literature³⁸

	Heinrichs et al (1990) ¹⁷	Hillyer et al (1992) ¹⁸	Boujon et al (1993) ⁶	Van der Kolk et al (1993) ⁴⁶	Couetil et al (1996) ⁸	Schott et al (2001) ³⁹
Hirsutism	47%	94%	100%	100%	95%	83%
Hyperhidrosis	NR	59%	67%	NR	14%	33%
Weight loss/ muscle wasting	NR	88%	NR	38%	50%	47%
Abnormal fat distribution	NR	12%	67%	19%	9%	29%
Lethargy	NR	82%	NR	NR	43%	NR
Chronic Laminitis	NR	82%	NR	24%	59%	52%
Polyuria/ Polydipsia	26%	76%	17%	NR	32%	34%
Chronic Infections	32%	48%	33%	NR	27%	NR
Neuro signs	21%	6%	50%	10%	NR	NR

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CHAPTER 2 - Pharmacokinetics of pergolide in normal mares

Introduction

Pituitary pars intermedia dysfunction (PPID) is a common metabolic disease that occurs in geriatric horses due to dopaminergic neurodegeneration.^{5,6,7,9} This disease, although more recently understood, has been diagnosed in horses for several decades. PPID typically occurs in horses over 15 years of age. The loss of hypothalamic dopaminergic neurons, which provide inhibitory control of the pars intermedia, results in an increase in production of pro-opiomelanocortin (POMC) peptides which include ACTH, α -MSH, β -END, and CLIP. The increase in this hormone/peptide production causes the common clinical signs associated with PPID which include hirsutism, PU/PD, immunosuppression, and laminitis.⁹ PPID can result in significant morbidity and mortality. Laminitis and immunosuppression often result in euthanasia of affected horses.

The treatment choice for PPID is pergolide, a dopamine agonist.⁹ However, there are no pharmacokinetic data about the use of this drug in horses. Pergolide was first used in human medicine for treatment of Parkinson's disease.^{1,10} The recommended dose and frequency of administration of pergolide to horses with PPID are largely based on clinical impression. The dose of pergolide used in horses ranges from 0.01 to 0.002 q 12 to 24 hours.⁹ Improvement in clinical signs, decreases in ACTH concentrations, and improvement in dexamethasone suppression tests are reported in horses with PPID treated with 0.002- 0.005 mg/kg of pergolide q 24 hours.^{2,9} Adverse effects associated with pergolide administration have been reported to be minimal in horses, in comparison to human complications.

While the use of pergolide is a logical approach for therapy in horses with PPID the pharmacokinetic and pharmacodynamic (PK-PD) relationship for suppression of POMC related peptides have not been established in the horse. Recent development in the field of high-performance liquid chromatography (HPLC)-tandem mass spectrometry assay has allowed for measurements of very low pergolide concentrations in plasma (range of pg/ml).^{3,4} This has allowed for some recent published reports of the pharmacokinetics of pergolide in humans. The first objective of this study was to provide pharmacokinetic data on the metabolism of pergolide in horse. A secondary focus of our study to determine the PK-PD effect of oral pergolide administration on ACTH and α -MSH in normal horses.

Materials and Methods

Animals- Seven healthy adult female horses were used in the study, which was approved by the Kansas State University Institutional Animal Care and Use Committee. The mares ages ranged from 3-17 years of age and breeds included 3 Quarter Horses, 3 Thoroughbreds, 1 Warmblood, and 1 Quarter Horse Cross. Body weight of the mares ranged from 355-582 kg. One horse was used in a pilot study to generate the initial pharmacokinetic data. The other six horses were used in the main pharmacokinetic-pharmacodynamic study. Physical examinations were within normal limits. There were no clinical signs of PPID, and haircoats were normal. An overnight dexamethasone suppression test was normal on all mares. Horses were housed in dirt paddock prior to the study, with prairie grass hay and water.

The mares were allowed free access in runs with shelter at all times other than during the study periods. The study was started at the end of June 2007. Rectal palpations with ultrasonography were performed to confirm ovulation 24-48 hours prior to enrollment in study period. At that time the mares were brought into an individual stall, weighed, and an intravenous (iv) jugular catheter placed. Mares remained in the individual stall for the entire study period of 6 days. Water, a pelleted diet, and ad libitum prairie grass hay were available at all times throughout the study period, except for the 8 hour fasting period prior to drug or placebo administration on day 1. All mares were accustomed to handling and blood collection was facilitated via the preplaced jugular catheter.

Drug Administration-

Pilot pharmacokinetic study: A single dose of 0.01mg/kg of pergolide was administered by mouth following an eight hour fast.

Pharmacokinetic-Pharmacodynamic study: Each of the six horses received drug (0.01mg/kg oral pergolide in molasses) or oral molasses after an eight hour fast on day 1. Doses were rounded up or down to the closest 0.5mg due to pergolide tablet formation being 1mg tablets that could easily be halved. Oral medications were administered by two investigators via a dosing syringe. 1mg pergolide tablets were obtained from a local human pharmacy for use in this study. Once a dose was calculated the tablets were crushed, placed into an oral dosing syringe, and then mixed with molasses.

Collection of samples and measurement of drug concentrations-

Pilot Pharmacokinetic Study: A single horse was brought into a stall the night before the study. An iv jugular catheter was placed. Blood samples were collected via a 14-gauge, 5.25-inch polyurethane catheter inserted into the right or left jugular vein, which was placed on day -1. An area over the jugular vein was clipped, aseptically prepared with chlorhexidine gluconate 4%, and rinsed with 70% isopropyl alcohol prior to infiltration of the skin with 2% lidocaine HCl in preparation for catheter placement. Jugular catheters were monitored for any signs of inflammation, if these signs did occur the catheter was removed and then replaced in the other jugular vein. The horse was administered 0.01mg/kg of pergolide by mouth following an eight hour fast. Blood to determine for pergolide concentrations was collected at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 7, 11, 16, 24, 36, 48, 60, 72, 84, and 120 hours in sodium heparin blood tubes. Blood samples were immediately placed on ice. Plasma was separated from red blood cells via refrigerated centrifuge. Plasma was stored in appropriately labeled polypropylene tubes at -70°C until analysis.

Pharmacokinetic-Pharmacodynamic study: In a randomized, blinded, cross-over design, six mares were either treated with pergolide or placebo. Horses were numbered from 1 to 6. Even numbered horses received pergolide in the first trial, and odd numbered horses received pergolide in the second trial. The collection of blood samples were performed by technicians unaware of what the horses had been treated with (pergolide or placebo). Horses were brought into an individual stall in the evening (day -1), and an iv jugular catheter was placed. Mares remained in their stalls for the entire study period (days -1, 0, 1, 2, 3, 4). There was a fourteen day washout period between either trial (placebo or pergolide).

Blood was collected from days 0 to 4, every 4 hours for ACTH and every 12 hours for α -MSH concentrations. Pergolide or placebo was administered on day 1 at 8:00 AM. Blood was also collected for pergolide, ACTH, and MSH concentrations at 0, 0.1, 0.25, 0.33, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72, and 96 hours after administration of pergolide or placebo. The time of blood collected for pergolide analysis was determined based on the data generated from the horse in the pilot pharmacokinetic study. Blood was collected in the same manner for control or treatment periods, and the amount of blood collected varied based the number of samples needed per sampling time. Blood was collected into EDTA tubes for hormone analysis and sodium heparin tubes for pergolide analysis. Blood samples were immediately placed on ice.

Plasma was separated from red blood cells within 30 minutes of collection via a refrigerated centrifuge. Plasma was placed into polypropylene tubes at -70°C for analysis.

Pergolide analysis: The analytical method for extraction of pergolide from plasma samples was a solid phase extraction method. It began by thawing the plasma then treatment with twice the volume of cold acetonitrile in order to precipitate proteins. 0.45 micron ultracentrifugation filter units were used to filter the sample into the desired analytical sample. Then 5-20 microliters (varied based on the expected pergolide concentration) were injected into the UPLC-MS/MS. Separation of the analyte occurred on an Xterra MS C₁₈, 5 micrometer, 2.1 x 150 mm column or equivalent with a mobile phase of acetonitrile/ammonium acetate (60:40, v/v) with a flow rate of 0.3mL/min. Once separation had occurred 10 microliters were injected into the UPLC/MS/MS. The lower limit of quantification was 0.5 ng/mL with a 10:1 noise to peak ratio. A correlation coefficient of 0.995 to known standards was obtained.

Hormone analysis: Plasma α -MSH concentrations were analyzed using a commercially available human radioimmunoassay, validated for use in horses, in duplicate. Assay sensitivity for α -MSH ranged from 2.17-2.62 pMol/L, intra-assay coefficient of variance ranged from 1.5-8.2%, and the inter-assay coefficient of variance was 7.81%. Plasma ACTH concentrations were analyzed in duplicate using a chemiluminescent enzyme immunoassay with a limit of quantification of 12 -1200pg/ml.

Statistical Analysis- Pharmacodynamic parameters (ACTH and α -MSH) were performed for treatment and control. Both hormones were evaluated using a multiple ANOVA for repeated measures comparing treatment, time, and period. This analysis was performed using JMP IN 5.1[®] (SAS Institute, Cary, NC) with statistical significance achieved when $p < 0.05$.

Pharmacokinetic analysis-- Once pergolide concentration verses time was determined for each mare, the data was entered into a commercially available program for non-compartmental pharmacokinetic analysis (WinNonLin[®] (Pharsight Corporation, Cary, NC)). The following pharmacokinetic parameters were calculated: AUC_{0-last}, elimination rate constant (k_{el}), terminal half-life ($t_{1/2el}$), total body clearance (corrected for unknown bioavailability) (CL_B/F), apparent volume of distribution (also corrected for unknown bioavailability) (V_d/F), maximal concentration (C_{max}), time to maximal concentration (T_{max}) and mean residence time. AUC_{0-last} was calculated using the linear trapezoidal rule and extrapolated to infinity by adding C_{last}/k_{el} .

K_{el} is the slope of the terminal portion of the time-concentration curve and the $t_{1/2el}$ was calculated as $0.693/k_{el}$. V_d/F and CL_B/F were calculated using standard equations.

Pharmacodynamic analysis- ACTH measurements and α -MSH measurements were evaluated using a multiple analysis of variance for repeated measures comparing treatment, time, and period. Analysis was performed using the commercially available software program JMP IN 5.1[®] (SAS Institute, Cary, NC). Statistical significance was considered with a p-value less than 0.05.

Results

Oral pergolide was tolerated well by 5 of the mares. One mare developed mild depression and anemia. The depression started at hour 3 after administration and continued until hour 32.

The pergolide concentrations for each horse at each time interval after oral administration are shown in table 2.1 and plotted in figure 2.1, 2.2, 2.3, 2.4, 2.5, and 2.6. The concentrations were below the level of quantification ($LOQ=0.5ng/ml$) at times 0, 24, and 48 for all horses. Estimates of the pharmacokinetic parameters using a noncompartmental analysis for each horse with mean and standard deviation are shown in table 2.2. The mean half life of oral pergolide in this study was 5.86 hours ($SD=3.42$ hours). The mean maximum concentration was $4.05 ng/ml$ ($SD=2.02 ng/ml$) with the median time to maximum concentration being 0.415 hours (Range: 0.33-1 hour). The mean $AUC_{0-\infty}$ was $14.08 h*ng/ml$ ($SD= 7.46 h*ng/ml$). The calculated apparent volume of distribution corrected for unknown bioavailability was $3,000 ml/kg$ ($SD=1,354 ml/kg$). The calculated apparent clearance corrected for unknown bioavailability was $1,204 ml/kg/hr$ ($SD=722 ml/kg/hr$). Bioavailability could not be calculated due to the lack of an available IV formulation for comparison. During the analysis of pergolide concentrations it was noted that there were two peaks during the absorption phase most likely attributable to biphasic absorption.

Mean ACTH concentrations for pergolide and placebo administrations are plotted in figure 2.7. Based on analysis there was no statistically significant difference found between treatment, time, or period in reference to ACTH concentrations. Mean α -MSH concentrations for placebo and control are plotted in figure 2.8. Analysis revealed a statistically significant

treatment by period interaction ($p < 0.02$). When evaluated separately the p-value for effect of period was 0.06 and the p-value for effect of treatment was 0.77.

Figures and Tables

Time (hrs) ID	0	0.1	0.25	0.33	0.5	0.75	1	1.5	2	3	4	5	6	8	12	24	48
1	<LOQ	0.94	3.13	4.83	3.45	2.95	4.15	1.44	1.49	1.22	1.0	0.81	0.73	0.59	<LOQ	<LOQ	<LOQ
2	<LOQ	0.51	0.76	0.96	1.3	0.95	2.11	0.93	0.91	0.66	0.61	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
3	<LOQ	0.63	1.76	2.52	2.46	1.78	2.07	1.59	1.12	0.91	0.72	0.57	<LOQ	0.53	<LOQ	<LOQ	<LOQ
4	<LOQ	1.70	4.43	6.04	5.17	6.20	4.48	2.88	1.49	1.54	0.98	0.90	0.74	0.77	0.60	<LOQ	<LOQ
5	<LOQ	<LOQ	1.51	2.17	2.22	1.96	2.18	0.76	0.68	0.62	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
6	<LOQ	1.45	2.91	6.42	5.67	5.65	4.13	2.29	1.85	1.20	1.14	0.89	0.68	0.55	<LOQ	<LOQ	<LOQ

Table 2.1: Pergolide concentration for each horse at sample time

	K_{el} (h⁻¹)	T_{1/2} (h)	C_{maz} (ng/ml)	AUC_{0-last} (h*ng/ml)	AUC_{0-∞} (h*ng/ml)	AUC % Extrapolated	MRT (h)	T_{max} (h)	Apparent Vd/F (ml/kg)	Apparent Cl/F (ml/kg/hr)
Horse 1	0.11	6.33	4.83	10.66	16.01	33.41	7.54	0.33	2432.72	781.64
Horse 2	0.19	3.65	2.11	3.69	6.90	46.58	5.25	1	5047.96	1921.36
Horse 3	0.22	3.10	2.52	7.53	9.89	23.83	5.20	0.33	4208.09	1080.26
Horse 4	0.06	12.39	6.20	16.40	27.16	39.62	14.07	0.75	1835.24	507.15
Horse 5	0.12	5.59	2.22	3.49	8.53	59.06	7.02	0.5	3301.73	2265.81
Horse 6	0.17	4.07	6.42	12.77	15.97	20.04	4.72	0.33	1663.27	669.64
Mean	0.15	5.86	4.05	9.09	14.08	37.09	7.30	0.415 (Median)	3,082	1,204
SD	0.06	3.42	2.02	5.15	7.46	14.56	3.50	0.33-1 (Range)	1,663-5,048 (Range)	507-2,269 (Range)

Table 2.2: Pharmacokinetic parameters for non-compartmental model for oral pergolide

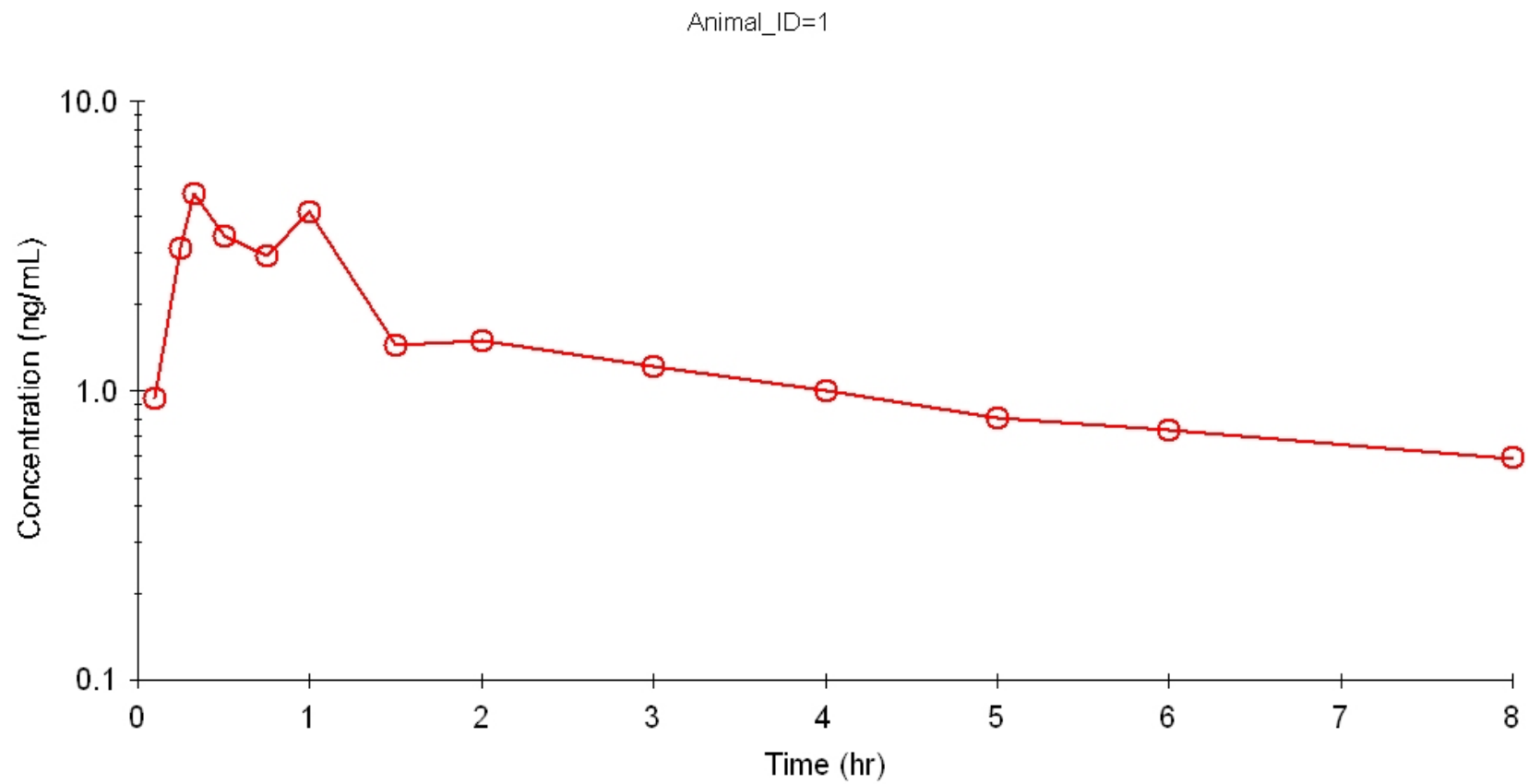


Figure 2.1: Pergolide concentration in Animal 1 verses time

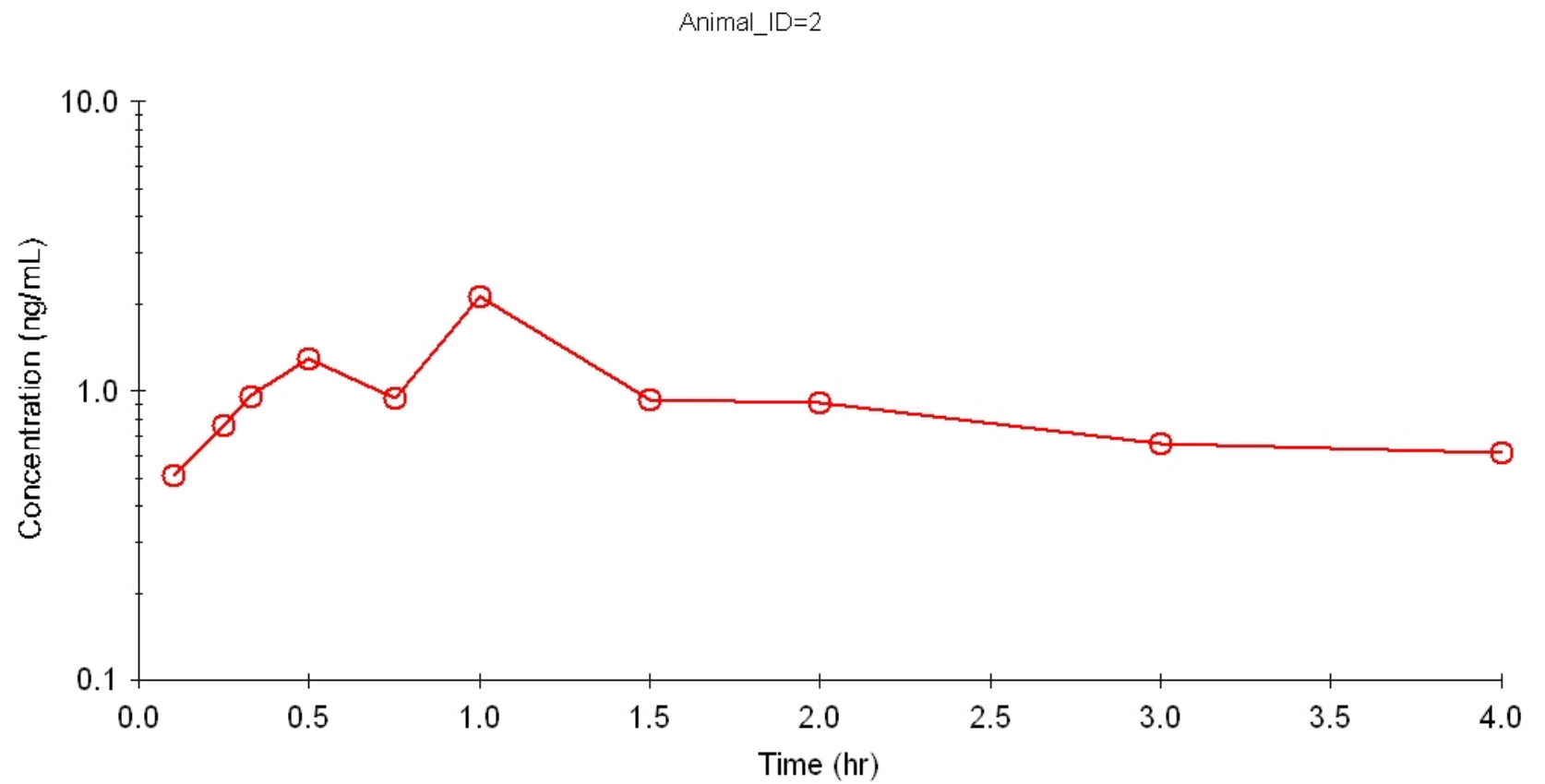


Figure 2.2: Pergolide concentration in Animal 2 verses time

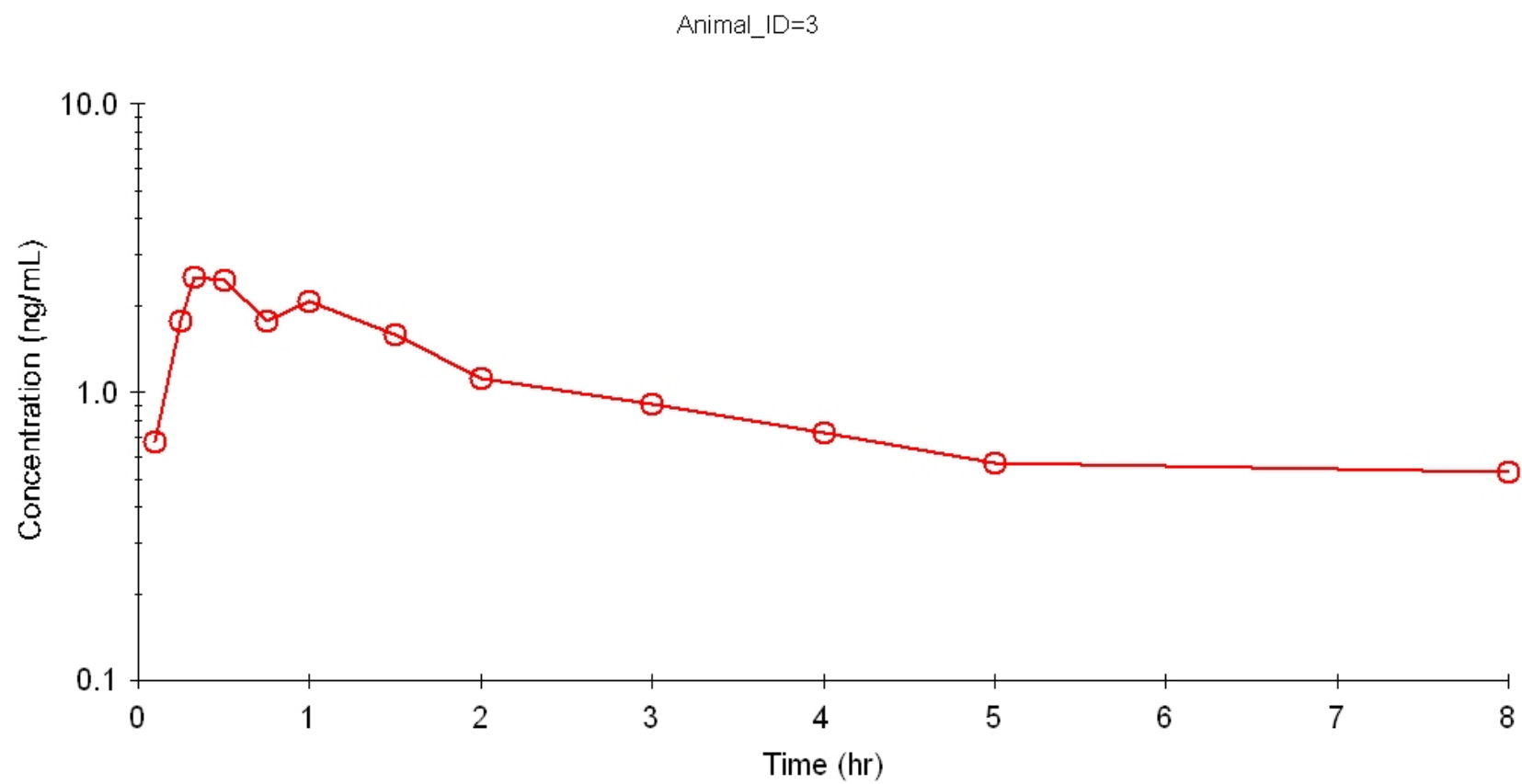


Figure 2.3: Pergolide concentration in Animal 3 verses time

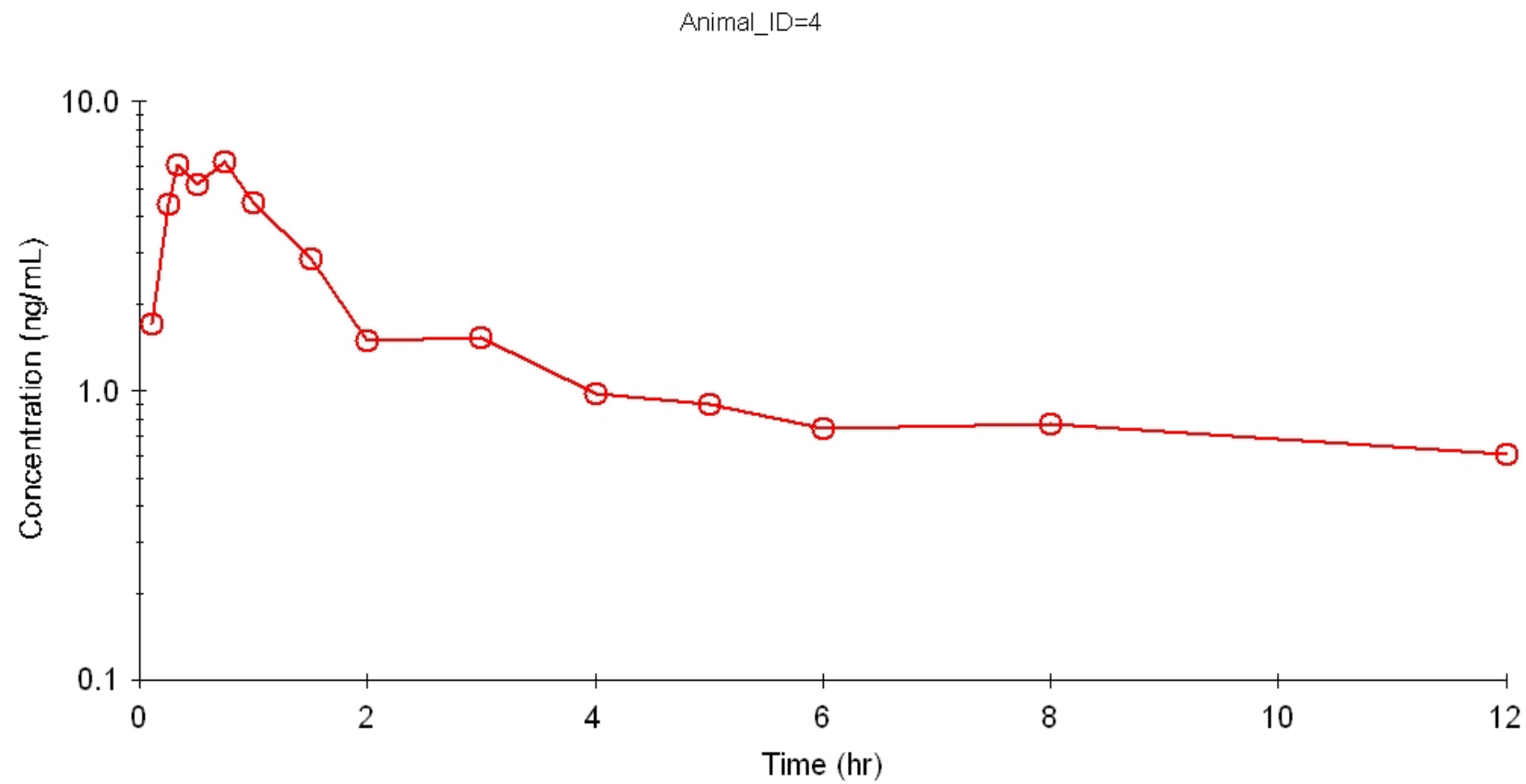


Figure 2.4: Pergolide concentrations in Animal 4 verses time

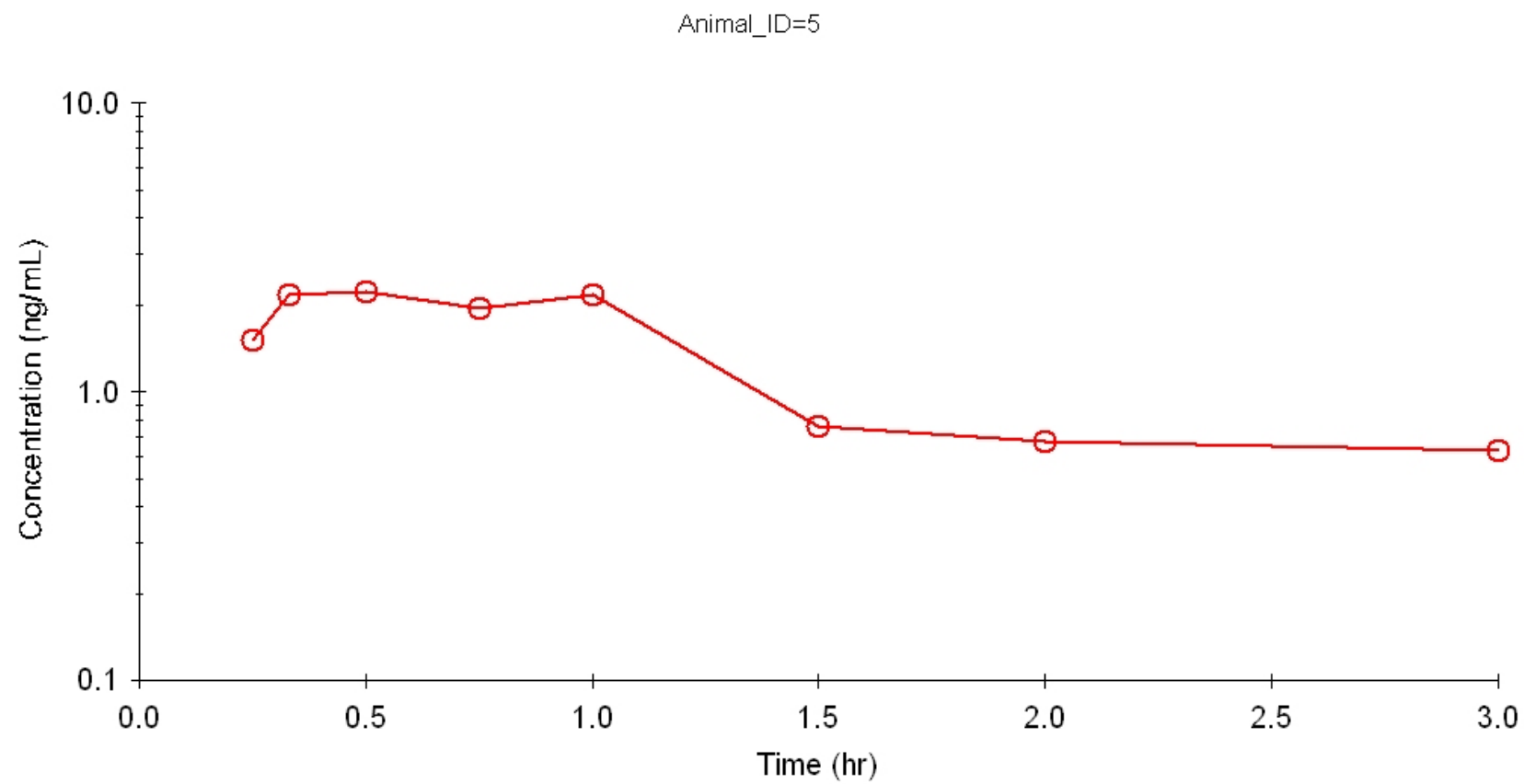


Figure 2.5: Pergolide concentrations in Animal 5 verses time

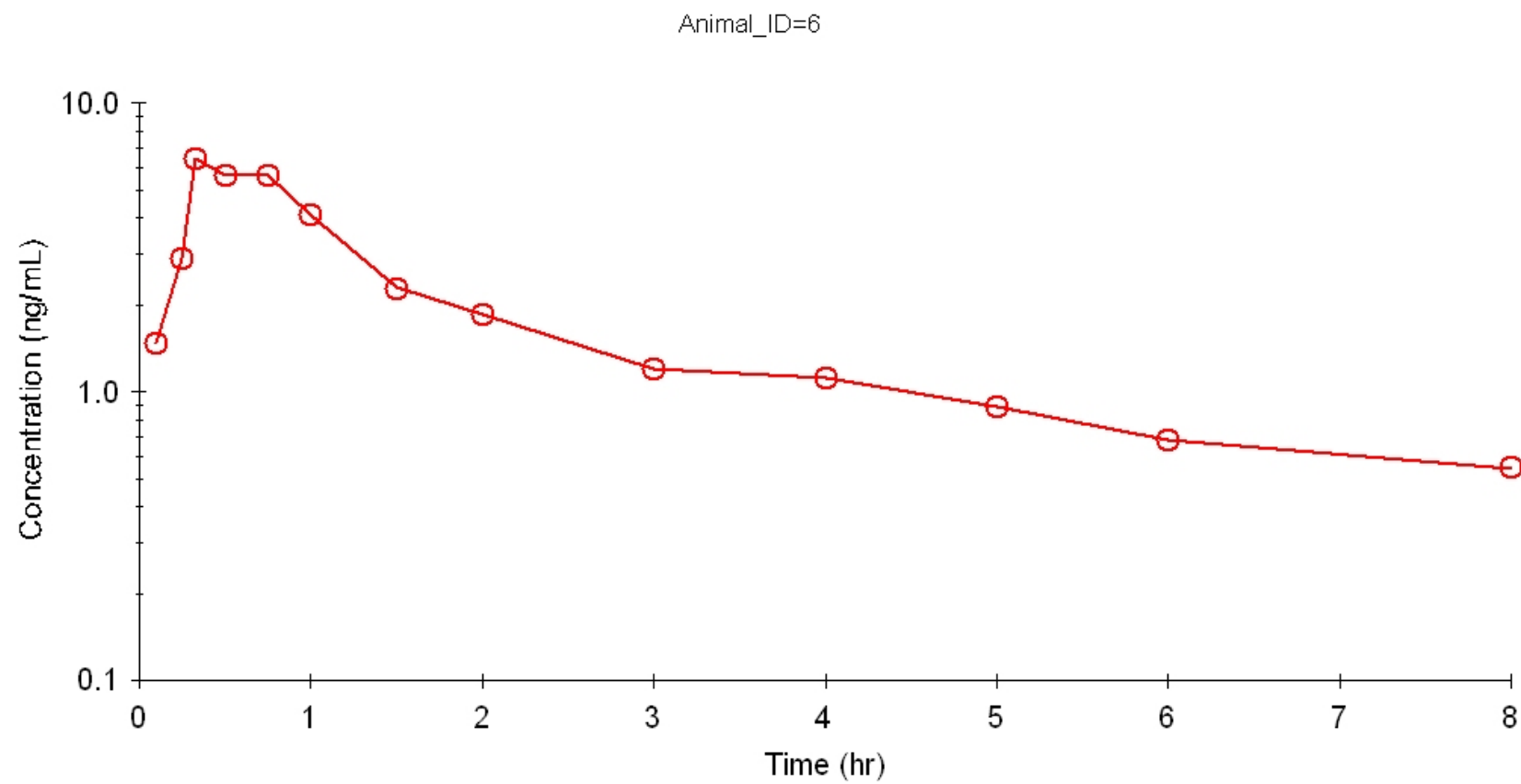


Figure 2.6: Pergolide concentrations of Animal 6 verses time

Plasma ACTH concentrations in horses treated with pergolide or placebo

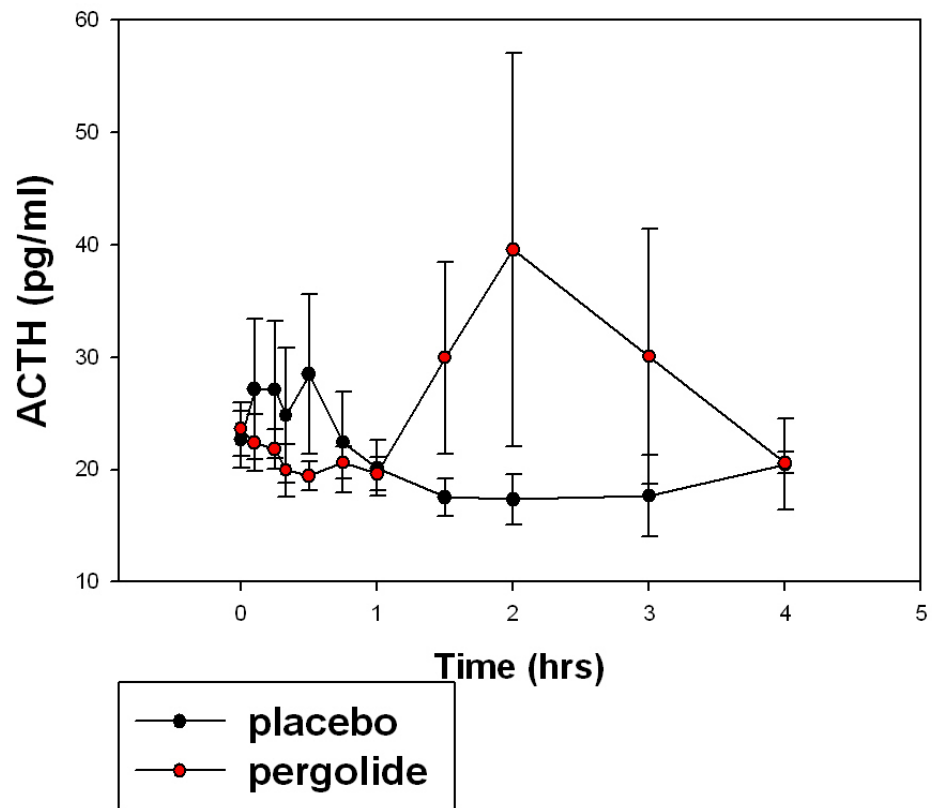


Figure 2.7: ACTH concentrations after pergolide or placebo administration

MSH (pmol/L) in placebo and pergolide treated horses

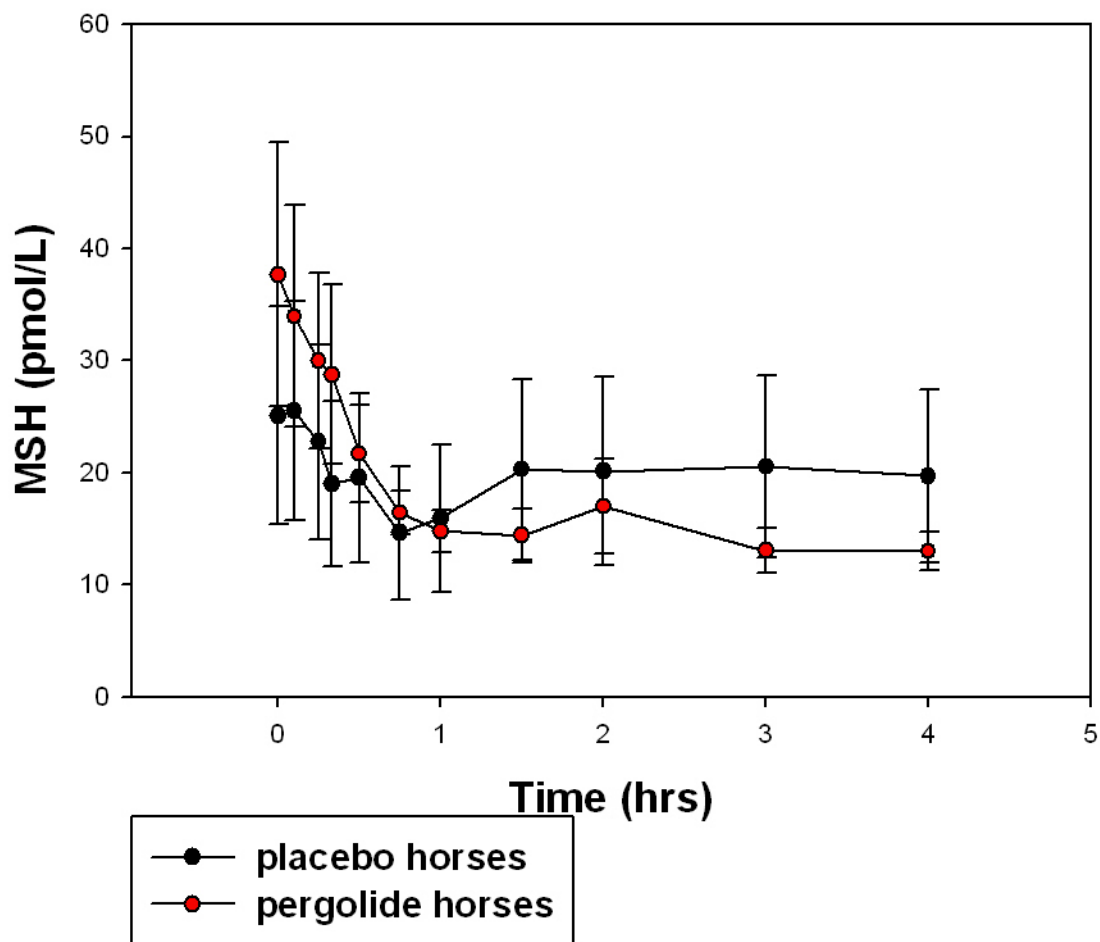


Figure 2.8: MSH concentrations for pergolide and placebo treated horses

Discussion

Pergolide has been used for many years to treat PPID in geriatric horses. There are several case series about the use of pergolide in affected horses; however none of these studies have actually evaluated the pharmacokinetics (or the PK-PD effects) of pergolide in horses. Our study documents that horses absorb pergolide when administered orally, and also provides some initial pharmacokinetic data.

The pharmacokinetic parameters obtained from non-compartmental analysis in our study indicate that horses absorb and clear pergolide much more efficiently than humans. The $T_{1/2}$ in our study was a mean of 5.86 hours; this is much shorter than the 21 hours found by Thalamas *et al.* in human patients.¹⁰ The time to maximum concentration in horses was also much shorter compared to humans, 0.415 hours compared to 2-3 hours. This may indicate that the interval between administrations may need to be shortened due to a shorter half life than expected. Bioavailability could not be determined due to the lack of an intravenous administration for comparison. The analytical method in our study had a higher lower level of quantification (0.5 ng/ml) than in the human studies (0.01 ng/ml).¹⁰ This higher level of quantification may have resulted in an under-estimation of the half-life due to missing of the slower terminal phase of elimination. The poor lower limit of quantification may be due to a difference in protein or lipid concentration in human versus horse plasma, an overly aggressive extraction method, or a lack of stability in frozen plasma. Performing the study at steady state and performing a different analytical method may help improve the lower limit of quantification if the experiment is performed again.

The pergolide dose used in this study was 0.01 mg/kg PO, which is much higher than the doses used clinically (range of 0.002 to 0.005 mg/kg). This study was a pilot project and the main objective was to determine the pharmacokinetics and PK-PD model of the suppressive effects of pergolide on POMC peptides. We chose a dose of 0.01 mg/kg based on the work performed by Orth *et al.* in 1982.⁸ This study reported that a single dose of pergolide (at 0.01 mg/kg) to a single horse resulted in a decrease in POMC peptide concentrations for 48 hours.⁸ In our study, this was not observed; however the Orth *et al.* study used a horse with PPID (thus having much higher POMC peptide concentrations).⁸ The higher dose was well tolerated by all

horses except one that became mildly depressed and anemic 3 hours after administration but was normal by hour 32. Pergolide administered at a range of 0.002 to 0.005 mg/kg q 24 hrs resulted in improved clinical signs and a decrease in ACTH concentrations.² Schott *et al.* reported that 7 out of 20 horses developed a normal dexamethasone suppression test when treated with pergolide at 0.002 mg/kg q 24 hours for 6 to 12 months.⁹

We were unable to document a PK-PD relationship on the suppressive effects of pergolide on POMC peptide concentrations. A statistical significant difference was not evident with ACTH concentrations. However, there was a significant treatment to period (or season) effect noted with α -MSH analysis ($p < 0.02$). When evaluating the effect of period ($p = 0.06$) and treatment ($p = 0.77$) separately, it is highly suggestive that the significance is more associated with the effect of period rather than treatment. We chose to conduct this study during the summer, when α -MSH concentrations would be moderately increased. Concentrations of α -MSH during the spring and winter in normal horses are close to the lower limits of sensitivity. Thus we were concerned that it might be difficult to measure the suppressive effects of pergolide during this time. Our particular study design (cross over design), with a 14 day wash-out period between trials, resulted in an increase in α -MSH concentrations during the second trial. An alternative would be to conduct each trial with a different set of horses, however this would likely result in increase variability and therefore the number animals needed to show significant differences.

The correct dose and frequency of administration of pergolide for treatment of PPID in horses is currently unknown, and our current study does not answer these questions. Further PK-PD studies on the suppressive effects of pergolide on POMC peptide concentrations should be performed to determine the most appropriate dose and dosing interval for treating PPID in horses. Ways to improve this study or improve the likelihood of obtaining better PK-PD information would include performing the study at a steady state, performing the study in a larger number of animals, performing the study when there is less effect of season on POMC peptide production, and performing the study in PPID affected horses. Horses with PPID have higher levels of POMC peptides and may have less variation due to season, this would allow for better evaluation of pergolide's effect on these peptides. Other than changing the season of the study, to execute these changes would increase the cost of the study significantly and finding a large number of PPID affected horses can be difficult at times.

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