PHARMACOKINETICS AND PHARMACODYNAMICS OF ORAL DEXAMETHASONE IN HEALTHY HORSES

by

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Abstract

Objective: To determine pharmacokinetic and pharmacodynamic properties of oral dexamethasone solution and powder compared to intravenous dexamethasone solution in healthy horses.

Animals: 6 horses, 13-27 years if age, 385-630 kg

Procedures: In a randomized, cross-over block design six healthy adult horses each received the following treatments 1) dexamethasone solution IV 0.05 mg/kg, 2) dexamethasone solution orally (PO) 0.05 mg/kg, and 3) dexamethasone powder PO 0.05 mg/kg all in the fed and fasted state. Each horse acted as an untreated control as secretion of cortisol was monitored for normal circadian rhythm. Quantification of plasma dexamethasone concentration and serum cortisol activity was determined by LC/MS and chemiluminescent enzyme immunoassay, respectively.

Results: Each horse exhibited a circadian rhythm in cortisol secretion; however there was variation present between each horse. Mean cortisol concentrations at 6:00 AM and 8:00 AM were significantly higher than concentrations at 8:00 PM and 10:00PM. Cortisol concentrations were significantly less than base-line starting 1 hour post-administration of dexamethasone through 72 hours for the fasted treatment groups, and 2 hours through 48 hours for the fed groups. Pharmacokinetic modeling resulted in a two compartment model for the IV administration with elimination from the central compartment, and a one compartment model for orally administered dexamethasone. Oral, fasted, compounded powder achieved a significantly higher maximum concentration (Cmax) than both fasted and fed oral dexamethasone solutions. The AUC_0^{inf} for the orally administered compounded powder was significantly different when comparing fasted versus fed treatment groups. Bioavailability ranged between 33% and 70% among treatment groups, but due to the high variability there was not a significant difference.

Conclusions and Clinical Relevance: Hospitalization of the horses did not have an effect on their circadian rhythm of cortisol secretion. Oral and intravenous administration of dexamethasone

resulted in adrenal suppression with cortisol concentrations returning to base-line 48-72 hours post-administration. Although bioavailability was variable cortisol suppression was similar among all treatment groups. The variability in oral absorption will need to be taken in to account for oral dosing of dexamethasone.

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Abbreviations for table 2.2:

λ_z = first-order rate constant. $t_{½}$ λ_z = half-life of the terminal portion of the curve. C0=Plasma concentration extrapolated to time 0. AUC_{all} = area under the curve from 0 to the last measured concentration. AUC_{inf} = area under the curve from 0 to infinity. AUC extrap. = % of the area under the curve from 0 to infinity extrapolated from the last time point. Vd_{area}=apparent volume of distribution of the area during the elimination phase. Vd_{ss}=apparent volume of distribution at steady state. Cl=plasma clearance. $AUMC_{all}$ = area under the first moment curve from 0 to the last measured concentration. $AUMC_{inf}$ = area under the first moment curve from 0 to infinity. MRT_{all} = mean residence time from 0 to the last measured concentration. MRT = mean residence time from 0 to infinity. F=extent of drug absorption compared to IV administration.

Abbreviations for table 2.3:

λ_z = first-order rate constant. $t_{½}$ λ_z = half-life of the terminal portion of the curve. C_{MAX} = Maximum measured plasma concentration. T_{MAX} = Time at which the maximum measured plasma concentration occurred. AUC_{all} = area under the curve from 0 to the last measured concentration. AUC_{inf} = area under the curve from 0 to infinity. AUC extrap. = % of the area under the curve from 0 to infinity extrapolated from the last time point. Vd_{area} /F=apparent volume of distribution of the area during the elimination phase per bioavailability. Cl/F =clearance per bioavailability. $AUMC_{all}$ = area under the first moment curve from 0 to the last measured concentration. $AUMC_{inf}$ = area under the first moment curve from 0 to infinity. MRT_{all} = mean residence time from 0 to the last measured concentration. MRT = mean residence time from 0 to infinity. F=extent of drug absorption compared to IV administration.

Abbreviations for table 2.4:

E_{MAX}=Maximum effect. ECe_{50}=Concentration required to produce 50% of the maximum effect. E_0=Effect at time 0. γ=shape parameter. Ke0=Rate of drug loss from the effect compartment. R=Correlation coefficient of model predicted versus measured parameter, V1=apparent volume of the central compartment. V/F=apparent volume of distribution per bioavailability. T_{LAG}=time lag from drug administration to drug absorption. K_{01}=rate of drug absorption to the central compartment. K_{10}=rate of drug elimination from the central compartment. K_{12}=rate of drug movement from the central compartment to compartment 2. K_{21}=rate of drug movement from compartment 2 to the central compartment.

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CHAPTER 1 - Literature Review

Adrenal Gland

Anatomy and Function

The adrenal glands in the horse are located at the medial-cranial poles of both the left and right kidney.[1,2] They are approximately 7-8 cm long, 3cm wide, 1.5 cm thick, and weigh approximately 15-20 grams.[2] The adrenal artery branches off of the aorta or renal artery to provide blood supply to the adrenal glands.[2] Sympathetic nerve fibers from the splanchnic nerve provide innervation to the adrenal glands.[2] Each adrenal gland is comprised of an adrenal medulla and an adrenal cortex. The adrenal medulla is centrally located and accounts for approximately 20% of the gland.[1] The adrenal medulla is closely related to the sympathetic nervous system as epinephrine and norepinephrine are released into circulation from the adrenal medulla in response to sympathetic stimulation.[1,2] The peripherally located adrenal cortex is further divided into three distinct zones: zona glomerulosa, zona fasciculata, and zona reticularis.[1] The zona glomerulosa is composed of a thin layer of cells that lie just beneath the capsule of the adrenal gland.[1] The zona glomerulosa comprises approximately 15% of the adrenal cortex, and is critical for secreting aldosterone in response to the renin-angiotensin system and changes in the osmolality of extracellular fluid.[1] The zona fasciculata lies just deep to the zona glomerulosa and comprises approximately 75 percent of the adrenal cortex.[1] Major hormones secreted from the zona fasciculata include cortisol and corticosterone in response to adrenocorticotropic hormone (ACTH) stimulation, in addition small amounts of androgens and estrogens are also secreted from the zona fasciculata.[1,2] The deepest layer of the adrenal cortex is the zona reticularis which is responsible for the secretion of the androgens dehydroepiandrosterone and androstenedione.[1] In addition, small amounts of estrogens and glucocorticoids are secreted from the zona reticularis.[1]

Glucocorticoids

Background

The primary endogenous glucocorticoid produced is cortisol which accounts for approximately 95% of glucocorticoid activity.[1,3] Corticosterone accounts for approximately 4% of glucocorticoid activity.[1,3] Cortisone has also been reported to be isolated from horses in small quantities.[4] The majority (80-95%) of cortisol in circulation is found in the inactive form bound to transcortin, a corticosteroid-binding globulin[1,5]. A smaller concentration can be found bound to albumin, and it is this albumin-bound portion that is able to diffuse extravascularly[5]. Plasma cortisol concentrations have been quantified using a variety of assays including radiostereoassay, thin layer chromatography, ultraviolet absorption, fluorescence, high pressure liquid chromatography, and radioimmunoassay. The half-life of cortisol has been reported to be 80 minutes in horses which is consistent with the half-life found in humans.[1,3] However, in another study performed by Slone et al, the half-life was found to be 2.1 ± 0.6 hours in bilaterally adrenalectomized horses.[6] In 1966, Zolovick et al, reported that mean combined cortisol and corticosterone concentrations in the horse ranged from 219.0 μg/dL to 395.3 μg/dL.[4] Since then various other studies, however have found either cortisol and corticosterone in combination or cortisol alone to measure 5.12 μg/dL, 1.37 μg/dL, 4.35 μg/dL, and 5.9 μg/dL.[7-10] Hoffsis et al, found mean cortisol concentrations to be elevated in cases of acute illness (shock, colic, fracture, dystocia, and anesthesia) ranging from 10.51 μg/dL to 16.4 μg/dL compared to 5.12 μg/dL in healthy horses.[11] Common synthetic analogues of cortisol used today in equine patients include dexamethasone, prednisolone, prednisone, methylprednisolone, triamcinolone, beclomethasone, and fluticasone. These synthetic glucocorticoids do not compete with endogenous cortisol for binding sites on transcortin and are more readily able to diffuse extravascullarly.[5] In addition, they have longer plasma and biological half-lives than cortisol.[5] Furthermore, synthetic glucocorticoids have increased anti-inflammatory potency when compared to endogenous cortisol. Prednisolone, methylpredisolone, and triamcinolone are 3-5 times more potent,

and dexamethasone and betamethasone are 20-30 times more potent than cortisol.[1,5] Table 1.1 lists cortisol and synthetic analogues with their anti-inflammatory potency and plasma and biological half-lives.[1,5] Because of these properties the synthetic glucocorticoids are able to have more rapid and prolonged biological effects than cortisol.[5] Melby reports that it is possible to approximate the duration of therapeutic effects of synthetic and natural glucocorticoids by evaluating the hypothalamic pituitary-adrenal suppressing activity.[5]

Table 1.1: Anti-inflammatory potencies and plasma and biological half-lives of cortisol and its synthetic analogues[5]

	Anti-inflammatory potency	Plasma half-life (min)	Biological half life (hr)
Cortisol	1	90	8-12
Prednisolone	3-5	200 or greater	12-36
Methylprednisolone	3-5	200 or greater	12-36
Triamcinolone	3-5	200 or greater	12-36
Betamethasone	20-30	300 or greater	36-54
Dexamethasone	20-30	300 or greater	36-54

Chemical Composition

Glucocorticoids (GC) are synthesized from cholesterol within the adrenal cortex.[1] Cholesterol enters the mitochondria where it is cleaved by cholesterol desmolase, and forms pregnenolone.[1] Within the mitochondria or endoplasmic reticulum pregnenolone is catalyzed by specific enzymes within various pathways resultling in the formation of aldosterone, cortisol, or androgens.[1] Both ACTH and angiotensin II hasten the conversion of cholesterol to pregnenolone.[1] Glucocorticoids have a four ring, 21-carbon structure (figure 1.1). The A ring contains a ketone group at C3 and a double bond at C4,5 which are essential for the anti-inflammatory properties of glucocorticoids.[12] It is the double bond associated with C1,2 that allows for an increase in

glucocorticoid activity without increased mineralocorticoid activity.[12] The C ring contains a hydroxyl group located at C11 that is critical for the anti-inflammatory activity of glucocorticoids, but again does not increase mineralocorticoid activity.[12] Inactive forms of glucocorticoids contain a ketone group instead of a hydroxyl group at C11. Following conversion to a hydroxyl group at the C11 position glucocorticoid activity is initiated.[12] Although synthetic glucocorticoids have primarily glucocorticoid activity; prednisolone, prednisone, methylprednisolone, cortisone, and isoflupredone have been shown to have mineralocorticoid properties as well.[1,13,14]

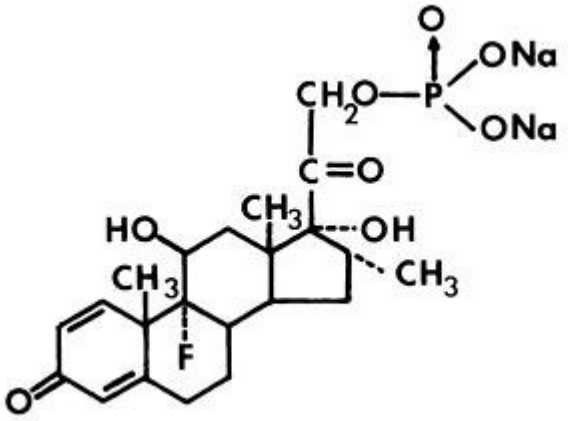

Figure 1.1: Chemical composition of dexamethasone

Metabolic Functions

Endogenous glucocorticoids secreted by the zona fasciculata have numerous metabolic functions that are essential for host health.[1,5,15,16] The primary glucocorticoid secreted from the adrenal cortex is cortisol composing 95% of glucocorticoid activity.[1] Glucocorticoids (primarily cortisol) gained their name for their ability to stimulate gluconeogenesis and maintain blood glucose concentrations.[1,5,15] Two main mechanisms of cortisol that stimulate gluconeogenesis are: 1) gene DNA transcription is activated by cortisol in the hepatocyte nuclei that leads to the production of proteins required for gluconeogenesis, and 2) cortisol enhances the mobilization of amino acids

making them readily available for gluconeogenesis.[1,15] In addition to increasing gluconeogenesis, cortisol decreases the rate at which glucose is utilized in the body, reducing the sensitivity of cells to insulin.[1,5,15,17] Peripheral insulin resistance is advantageous to the host because this maintains the availability of glucose for the central nervous system and heart, which are organs that do not require insulin for uptake of glucose. The mechanisms through which these mechanisms occur are not completely understood. Cortisol has been shown to decrease protein synthesis and increase catabolism of protein in extra-hepatic tissues.[1,5] Since cortisol suppresses the transport of amino acids into extra-hepatic tissues, and the catabolic effects of cortisol results in release of amino acids from these extra-hepatic tissues amino acids are available for enhanced hepatic protein production.[1,5] Cortisol also promotes lipolysis through the mobilization of fatty acids from adipose tissue.[1] Again the mechanisms by which cortisol regulates these metabolic functions remains to be fully elucidated.

Stress

Cortisol secretion is significantly increased during stressful and painful situations. There are several types of stress that have been related to cortisol secretion including: trauma, infection, intense heat or cold, injection of sympathomimetic drugs, surgery, restraint, and debilitating disease.[1,15] It is well understood that enhanced secretion of ACTH from the anterior pituitary increases the secretion of cortisol.[1,15] However, the benefit of the enhanced secretion is not well understood. Several theories have been proposed including the mobilization of amino acids and fats making them accessible for energy and synthesis of other compounds.[1,15]

Anti-inflammatory and Immunosuppressive Effects

The anti-inflammatory and immunosuppressive properties of glucocorticoids are the primary reason for their clinical use. Cortisone, the inactive form of cortisol, was first isolated in 1936, and Reichstein first synthesized cortisol in 1938.[18] In 1950, Hench and coworkers received the Nobel Prize for describing the beneficial effects of exogenous glucocorticoids for the treatment of

osteoarthritis in human patients.[12,18,19] Glucocorticoids are used for a variety of conditions in equine patients including: recurrent airway obstruction, inflammatory airway disease, dermatitis, purpura hemorrhagica, neurological disease and trauma, shock, arthritis, neoplasia, and immune-mediated conditions.[15,20-22]

Glucocorticoids suppress inflammation and exert immunosuppressive effects through a variety of mechanisms. Prior to the current understanding of the mechanisms associated with the anti-inflammatory effects of glucocorticoids certain cellular characteristics were recognized. Glucocorticoids have been recognized to have multiple properties in preventing inflammation such as: 1) stabilization of lysosomal membranes; 2) decreasing the permeability of capillary walls; 3) suppression of migration of white blood cells into areas of inflammation and decreased phagocytosis of damaged cells; 4) immunosuppression associated with decreased T-lymphocyte production/survival; as well as 5) suppressing the release of IL-1 or the binding of IL-1 to its receptors to aid in attenuating fever.[1,5,23] The most important effects of glucocorticoids on different cell types can be found in Table 1.2.[18,23-26]

Table 1.2: Glucocorticoid effects on primary and secondary immune cells[27]

Monocytes/macrophages
↓ Number of circulating cells (↓ myelopoiesis, ↓ release)
↓ Expression of MHC class II molecules and Fc receptors
↓ Synthesis of pro-inflammatory cytokines (e.g. IL-2, IL-6, TNFα) and prostaglandins
T cells
↓ Number of circulating cells (redistribution effects)
↓ Production and action of IL-2 (most important)
Granulocytes
↓ Number of eosinophils and basophil granulocytes
↑ Number of circulating neutrophils
Endothelial cells
↓ Vessel permeability
↓ Expression of adhesion molecules
↓ Production of IL-1 and prostaglandins
Fibroblasts
↓ Proliferation
↓ Production of fibronectin and prostaglandins

It has been a long accepted theory that glucocorticoids exert their anti-inflammatory properties by inhibiting the action of phospholipase A_2. This theory was challenged by Lane et al, which monitored the anti-inflammatory effects of systemic dexamethasone on exudate concentrations of the eicosanoids prostaglandin E_2, thromboxane B_2, 6-keto-$PGF_{1\alpha}$, and leukotriene B_4.[28] In their study, dexamethasone revealed no significant suppression of exudate concentrations of the previously mentioned eiconsanoids.[28] Therefore, it was concluded that the primary anti-inflammatory property of glucocorticoids was not through the inhibition of phospholipase A_2.[28] Even though that study failed to demonstrate suppression of eicosanoids in exudate, associated with the administration of dexamethasone, it was shown that prostaglandin, arachidonic acid, and other inflammatory mediators were inhibited by glucocorticoids.[29,30] Current understanding of glucocorticoids divides their actions into classical genomic effects and non-genomic effects.[12,18,19,29,30]

The classical genomic properties are mediated by the cytosolic glucocorticoid receptor (GCR), a 94-kD protein. The GCR exists as a multiprotein complex with a variety of heat shock proteins, such as Hsp90, Hsp70, Hsp56, and Hsp40.[18] The GCR also has interaction with immunophilins, (co)chaperones, and kinases of the mitogen-activated protein kinase (MAPK) signaling system.[18] The GCR is comprised of three different domains with a variety of functions: an N-terminal domain containing transactivation functions, a DNA-binding domain, and a ligand-binding domain that consists of 12 α-helices and is involved in the formation of the hydrophobic ligand-binding pocket.[18] Because of their lipophilic structure, glucocorticoids are able to easily enter the cytosol of the cell through passive diffusion.[12,18] Glucocorticoids bind to the GCR resulting in activation of the GC/GCR receptor complex, with subsequent dissociation of the (co)chaperones from the GC/GCR complex occurring.[12,18] Within 20 minutes of the (co)chaperone dissociation the GC/GCR complex is translocated into the nucleus where it binds to glucocorticoid responsive elements (GREs).[12,18,19] Once the GC/GCR has entered the nucleus gene transcription can be impacted. The binding of the activated GC/GCR complex to positive GREs induces the synthesis of

anti-inflammatory proteins (lipocortin 1 and IκB).[12,18,19] Conversely glucocorticoids can inhibit gene transcription via binding between GCR and negative GREs. Down regulation of protein synthesis has been shown to suppress transcription of the inflammatory genes interleukin-1 and interleukin-2.[18,30] Glucocorticoids are also able to affect transcription of genes that do not contain GREs, and inhibit pro-inflammatory gene expression through transrepression.[12,18,19,30] This property occurs when the GC/GCR complex interacts directly or indirectly with transcription factors such as activator protein 1 (AP1), nuclear factor- κB (NF-κB), or interferon regulatory factor-3 (IRF-3).[12,18,19,30] These transcription factors are involved in regulating the expression of pro-inflammatory genes by reducing transcriptional activities resulting in anti-inflammatory and immunosuppressive effects.[12,18,19,30] Through this negative regulation, glucocorticoids prevent the translocation and function of pro-inflammatory transcription factors, thus suppressing synthesis of inflammatory mediators such as IL-1, IL-2, IL-6, IL-8, TNF-α, IFN-γ, and prostaglandins.[12,18,19,30] The principle mechanism for this negative regulation is through the synthesis of IκB.[12,18,19,30] Within the cytoplasm of inactive cells, NF-κB is bound to an IκB protein.[12,19,30] When bound to NF-κB, IκB prevents the translocation of NF-κB into the nucleus.[12,19,30] Once stimulated, IκB undergoes phosphorylation and degradation allowing NF-κB to enter the cell nucleus and interact with genes responsible for IL-1, IL-2, IL-6, IL-8, TNF-α, IFN-γ, and prostaglandins.[12,18,19,30] Mechanisms that do not involve GREs are also critical for controlling the pro-inflammatory cellular effects. These mechanisms include 1) binding of the GCR to the p65 subunit of the NF-κB domain thus inhibiting the transcriptional activity of NF-κB; 2) inducing synthesis of IκB which chelates activated NF-κB and blocks transcriptional activity of NF-κB; and 3) competition of coactivators between the GC/GCR complex and various transcription factors.[12,18,30] The genomic effects of glucocorticoids usually take hours to days to become evident.

Rapid clinical responses are commonly seen with glucocorticoid administration that cannot be explained by the classical, genomic mechanisms of glucocorticoids. Therefore, several different non-genomic mechanisms have been hypothesized for these properties. One suggested theory

involves non-specific glucocorticoid interactions with cellular membranes that alters the physiochemical properties and activities of membrane-bound proteins. This mechanism results in decreased sodium and calcium transport across the plasma membranes of immune cells.[18,29] Subsequently this is believed to result in rapid immunosuppression and a decrease in the inflammatory process.[18,29] Another hypothesis that may explain the rapid effects of glucocorticoids is that glucocorticoids bind to GCRs and not only result in classical genomic effects, but also rapid non-genomic effects. The underlying theory is that release of arachidonic acid from cell membrane-associated phospholipids is regulated by mediators which includes growth factors, adaptor proteins, MAPK, phospholipase A2, and lipocortin 1.[18,29] It has been shown that dexamethasone can inhibit the release of arachidonic acid subsequent to phospholipase A2 activation through a GCR-dependent, but a transcription-independent mechanism.[18,29] The final hypothesis that may justify non-genomic glucocorticoids effects on immune cells involves the presence of a membrane-bound GCR which is a variant of the cytosolic GCR associated with genomic effects.[18,29] It has been shown that immunostimulation increases the percentage of membrane-bound GCR which may indicate that they play a role in chronic inflammatory disease.[18,29] Regardless of the mechanism, rapid non-genomic properties play an essential role in the control of inflammation and immunosuppression.

Side Effects of Glucocorticoids

Systemic glucocorticoid administration in the equine host has been associated with adverse side effects such as hypothalamic-pituitary-adrenal axis suppression, muscle wasting, hyperglycemia, polyuria, polydipsia, immunosuppression, and laminitis.[15,17,31,32] Adrenocortical dysfunction can be monitored through endogenous cortisol response to ACTH administration. Multiple studies have investigated the horse's adrenocortical function in response to exogenous glucocorticoids. Cortisol suppression, indicating adrenal suppression, occurs following intravenous, intramuscular, and aerosolized formulations of glucocorticoids.[13,21,31-34] It appears that short-term parenteral

administration of dexamethasone and prednisolone sodium succinate do not result in adrenocortical dysfunction as they are still responsive to ACTH stimulation testing.[21,31,33,34] However, a single dose of prednisolone acetate and triamcinolone has resulted in adrenocortical dysfunction for 14 to 21 days.[14,21,36,37] Anecdotal evidence suggests an association between glucocorticoids and equine laminitis, however a cause and effect relationship has yet to be demonstrated. Despite the suggestion, the pathophysiology of equine laminitis in association with glucocorticoid administration is under investigation, but is beyond the scope of this thesis.[15,17]

Cortisol Secretion

Regulation of Cortisol Secretion

Cortisol secretion from the adrenal cortex is mediated by ACTH released from the anterior pituitary. Initially, corticotropin-releasing factor (CRF) is secreted from the hypothalamus, and is carried to the anterior pituitary via capillary plexus of the hypophysial portal system. Upon binding of CRF to CRF receptors in the anterior pituitary ACTH secretion is stimulated.[1,16] ACTH then acts upon the adrenocortical cells and activates adenylyl cyclase in the cellular membrane. The activation of adenylyl cyclase stimulates cAMP formation within the cytoplasm.[1,16] The formation of cAMP then results in the activation of intracellular enzymes essential for the production of adrenocortical hormones.[1,16] It is the formation of cholesterol desmolase that is essential for the conversion of cholesterol to pregnenolone, which is the "rate-limiting" step in adrenocortical hormone production.[1,16] The formation of CRF in the hypothalamus and the formation of ACTH in the anterior pituitary are controlled by a direct negative feedback of cortisol.[1,16]

Circadian Rhythm of Cortisol Secretion

The circadian rhythm of plasma glucocorticoid secretion has been documented in humans, rhesus monkeys, rats, dogs, mice, channel catfish, and swine.[1,4,8,10] Some studies performed in the horse have been successful in identifying a circadian rhythm while others have not. Hoffsis et al,

were unable to appreciate a consistent cortisol pattern in horses that underwent sampling at two and four hour intervals.[7] However, a consistent pattern in the same horses was observed when sampling was performed at 28 hour intervals with concentrations peaking at approximately 8:00 am and reaching trough concentrations at approximately 4:00 pm.[7] Because the half-life of cortisol is approximately 100 minutes, it was speculated that any effect venipuncture had on cortisol secretion would be minimal by 24 hours later.[7] In that study, the effect of venipuncture and frequency of sample collection was believed to be responsible for diminishing the circadian rhythm.[7] Another study performed by Bottoms et al, identified a rhythm in horses that underwent sample collection via venipuncture every two hours with peak and trough concentrations occurring at 8:00 am and 10:00 pm, respectively.[8] Toutain et al, performed hourly sample collection via jugular catheter to monitor cortisol secretion.[9] Their analysis revealed an episodic pattern with intermittent peaks and troughs.[9] However, they did recognize a consistent peak at 6:00 am and trough concentrations from 9:00 pm to 11:00 pm.[9] Irvine et al, analyzed circadian rhythm in a variety of environments and management practices.[10] They found untrained horses in their normal environment and trained race horses maintained in their normal environment and daily routine had peaks between 6:00 am to 9:00am, and troughs between 6:00 pm to 9:00 pm.[10] Furthermore they found that the same untrained horses housed in a novel environment did not exhibit a circadian rhythm, and had a higher mean plasma cortisol concentrations when compared to those who exhibited a circadian rhythm.[10] Findings from these studies strongly suggest that a circadian rhythm exists in horses, but is fragile and can be easily disrupted.

Pharmacodynamic/Pharmacokinetic Modeling

Pharmacodynamics of Glucocorticoids

Pharmacodynamic studies have been performed including those evaluating the effect of glucocorticoids and adrenal gland function on leukocyte counts, plasma electrolyte concentrations,

cortisol secretion, pulmonary lung function, and cytologic evaluation of bronchoalveolar lavage (BAL) fluid. Some of the earliest studies, dating back to 1948, focused on the effects of glucocorticoids on circulating eosinophils.[7,11,25] Suppression of circulating eosinophils was determined to be an insensitive and unquantifiable tool for evaluating glucocorticoids effects on adrenal function.[7,11,25] In addition to eosinophil response to glucocorticoids, evaluation of other leukocytes became the focus. These studies revealed a significant leukocytosis and eosinopenia four hours after the administration of corticosteroids that lasted up to 17 hours.[24,25] Furthermore, lymphopenia was also recognized for up to 16 hours following intramuscular dexamethasone administration.[24,25] To further evaluate the effects of glucocorticoids on adrenal function, Eiler et al, monitored the effect corticosteroids had on plasma concentrations of sodium, potassium, calcium, and magnesium.[25] Their work revealed that there was no significant effect on plasma sodium concentration, however there was significant elevations in calcium, magnesium, and potassium four hours after dexamethasone administration.[25] They concluded that this was a direct effect of dexamethasone related to its catabolic effects on muscle and bone.[25] Various studies have evaluated the effects of corticosteroids on cortisol secretion. Many of the early studies examined cortisol suppression in conjunction with leukocyte count and electrolyte concentrations following IM administration of dexamethasone.[7,25] Hoffsis et al, administered dosages of dexamethasone ranging 2-80 milligrams, and appreciated suppression of cortisol in all treatment groups. Maximal suppression occurred between 12 and 24 hours, and returned back toward baseline by 72 hours for all treatment groups.[7] Eiler et al, administered 20 milligrams of dexamethasone IM and monitored plasma cortisol concentrations for eight hours with maximal suppression occurring at approximately 6 hours post dexamethasone administration.[25] In that study they found maximal suppression of cortisol to be similar to the time of maximal change in electrolyte,leukocyte and eosinophil concentration.[25] These studies were critical in establishing the effects that dexamethasone had on adrenal suppression in equine patients. Studies subsequently began to compare the effects of

dexamethasone and other corticosteroids on adrenal suppression. Toutain et al, evaluated the effects of dexamethasone alcohol, dexamethasone 21-isonicotinate, prednisolone 21-sodium succinate and prednisolone acetate on adrenal suppression.[21] After IV administration of both dexamethasone formulations, plasma cortisol concentrations were significantly decreased from baseline two hours post-administration and remained suppressed for 72 hours.[21] These findings were further supported by Soma et al, who found significant cortisol suppression from baseline beginning one hour following 0.05 mg/kg IV administration of dexamethasone that persisted for 72 hours.[20] Following IM administration of both dexamethasone formulations similar results were seen, but cortisol remained suppressed until five days post-administration.[21] With regards to prednisolone 21-sodium succinate, IV administration resulted in cortisol suppression 4 minutes post-administration, and IM administration resulted in suppression 9 minutes following administration.[21] Cortisol concentrations returned to baseline values at 24 hours. In contrast, the IM prednisolone acetate resulted in cortisol suppression at two hours, but remained suppressed until 21 days post-administration.[21] Rush et al, compared aerosolized beclomethasone diproprionate and intravenous dexamethasone in horses with induced recurrent airway obstruction.[22,32] Both the aerosolized beclomethasone and parenteral dexamethasone resulted in significant suppression of cortisol within two days of administration.[22,32] Cortisol concentrations returned to values similar to control horses two days after discontinuation of the beclomethasone and four days after discontinuing the dexamethasone.[22,32] Cortisol suppression associated with the aerosolized beclomethasone was a surprising finding since human patients rarely experience the systemic effects of aerosolized beclomethasone.[22,32] The observation of cortisol suppression in response to aerosolized beclomethasone indicated the equine host systemically absorbs aerosolized beclomethasone. This may further indicate that horses have increased sensitivity to corticosteroids than human patients. The mainstay of research today surrounds the therapeutic effects of corticosteroids for recurrent airway obstruction. Multiple studies have evaluated the effects of dexamethasone, beclomethasone, and prednisone on pulmonary function and cytological

evaluation of BAL fluid. Improved pulmonary function was observed when aerosolized beclomethasone, intravenous, intramuscular, and oral dexamethasone were administered.[33,35,38-41] Recently, DeLuca et al, observed reduced expression of IL-8, chemokine ligand 2 and IL-1β in bronchoalveolar lavage fluid following the administration of oral dexamethasone.[41] The combination of improved pulmonary function and cortisol suppression following administration of synthetic corticosteroids provides support that adrenal suppression may approximately parallel the anti-inflammatory effect and metabolic half-life.[5,31] Prednisone administered orally has not been shown to have consistent beneficial effects for horses affected with recurrent airway obstruction.[38,39,42] This is most likely due to decreased absorption of prednisone and decreased metabolism to the active drug prednisolone.[38,39,42] Recently Soma et al, also evaluated the effects of dexamethasone on plasma glucose and lactate concentrations.[20] Following IV dexamethasone administration, significant increases in lactate concentration and glucose were present between 4 and 60 hours, 8 and 36 hours, respectively.[20] These changes highlight the influence glucocorticoids have on carbohydrate metabolism and subsequent gluconeogenesis.[20]

Pharmacokinetics of Glucocorticoids

Few pharmacokinetic studies have been performed in the equine host. Utilizing HPLC with a level of sensitivity of 2 to 3 ng/ml, Toutain et al, was the first to describe pharmacokinetic parameters for dexamethasone in horses.[21] In that study dexamethasone was detectable in plasma for up to 150 to 180 minutes post administration, and a two-compartment open model with elimination from the central compartment best described the elimination of dexamethasone from plasma.[21] In addition, the concentration of dexamethasone at time 0 (C_p^o) was 346.4 ng/ml for dexamethasone alcohol and 301.25 ng/ml for dexamethasone 21-isonicotinate following a 0.05 mg/kg dose intravenously.[21] Complete pharmacokinetic parameters for IV dexamethasone alcohol and dexamethasone 21-isonicotinate can be found in table 1.3.

Table 1.3: Mean pharmacokinetic parameters of dexamethasone following an IV bolus of dexamethasone alcohol or dexamethasone 21-isonicotinate[21]

Pharmacokinetic	Mean ± SD IV dexamethasone alcohol	Mean ± SD IV Dexamethasone 21-isonicotinate
A (ng/ml)	302 ± 83.8	251.8 ± 123.8
B (ng/ml)	44.25 ± 8.8	49.4 ± 8.87
α (min^{-1})	0.45 ± 0.12	0.48 ± 0.21
β (min^{-1})	0.01 ± 0.005	0.01 ± 0.004
k_{12} (min^{-1})	0.31 ± 0.11	0.33 ± 0.18
k_{21} (min^{-1})	0.07 ± 0.006	0.09 ± 0.17
k_{el} (min^{-1})	0.09 ± 0.01	0.07 ± 0.03
V'_c (ml/kg)	151.3 ± 35.4	189.3 ± 66.8
V'_d(area) (ml/kg)	966 ± 192.6	906.5 ± 188.4
Cl_B (ml/min*kg)	12.8 ± 2.25	12.3 ± 3.77
$t_{1/2(B)}$ (min)	53.3 ± 14.0	53.6 ± 17.08

In 1996, Cunningham et al, was the first to compare pharmacokinetic properties of intravenous dexamethasone to oral dexamethasone powder.[43] Using radioimmunoassay with a sensitivity of 100 pg/ml, maximum serum concentrations of dexamethasone were 23,200 pg/ml and 4,900 pg/ml following administration of 10 mg intravenously and 10 mg orally, respectively.[43] The $t_{1/2}$ was considerably longer in this study for both the IV administration (2.63 hours) and oral administration (4.36 hours) when compared to those in the Toutain study.[21,43] Following both oral and IV administration, serum dexamethasone concentrations could be detected in the majority of horses at 12 hours with a few having detectable levels at 24 hours.[43] These findings are consistent with the improved sensitivity of the radioimmunoassay utilized. The bioavailability for the oral dexamethasone powder was determined to be incomplete and variable (31%-88%) with a mean of 61%.[43] Complete pharmacokinetic parameters following 10 mg dexamethasone solution IV and 10 mg dexamethasone powder orally can be seen in table 1.4.

Table 1.4: Mean pharmacokinetic parameters for IV and oral dexamethasone following a 10 mg dose

Pharmacokinetic	Dexamethasone 10 mg IV	Pharmacokinetic	Dexamethasone Powder 10 mg orally
α (h^{-1})	3.15 ± 3.38		
β (h^{-1})	0.26 ± 0.11	β (h^{-1})	0.16 ± 0.05
AUC (ng*h/ml)	47.9 ± 6.44	AUC (ng*h/ml)	29.09 ± 8.69
Vc (L/kg)	0.99 ± 0.33	C_{max} (pg/ml)	4900 ± 170
Vss(L/kg)	1.73 ± 0.48	t_{max} (h)	1.3 ± 0.5
Cl (L/h*kg)	0.48 ± 0.06	F	0.61 ± 0.19
$t_{1/2}$ (h)	2.63 ± 1.19	$T_{1/2}$ (h)	4.36 ± 1.34

Pharmacokinetic analysis of prednisolone revealed that oral tablets had a bioavailability of 65% and oral liquid to have a bioavailability of 56%.[39] These findings in addition to Cunningham et al, have been the hallmark for oral bioavailability and oral dosing of glucocorticoids.[33] Soma et al, utilized liquid chromatography interfaced with triple spray quadrupole quantum tandem mass spectrometry to completely quantify dexamethasone, cortisol, and cortisone following IV administration of dexamethasone.[20] Plasma concentrations were estimated to be 65.6 ± 21.6 ng/ml at C_p^o.[20] Because of the increased sensitivity they were able to determine a three compartment model best described the elimination of dexamethasone.[20] Elimination half-lives for each compartment were 0.33 hours, 2.19 hours and 10.7 hours, and dexamethasone was still detected in two of the six horses at 48 hours post-administration.[20] However, at 72 hours post-administration the plasma concentrations of dexamethasone were below the level of quantification (LOQ) for all horses.[20] All of the pharmacokinetic studies have been beneficial in determining the pharmacological effects of dexamethasone, but it appears Soma et al, have provided a complete explanation of the

pharmacokinetics following IV administration. Median pharmacokinetic parameters may be found in table 1.5.

Table 1.5: Pharmacokinetic parameters of dexamethasone following 0.05 mg/kg IV administration

Pharmacokinetic	Median	Range
α (h^{-1})	2.25	1.52-4.24
$t_{1/2\alpha}$ (h)	0.33	0.22-0.57
β (h^{-1})	0.32	0.26-0.37
$t_{1/2\beta}$ (h)	2.19	2.12-2.66
γ (h^{-1})	0.07	0.05-0.10
$t_{1/2\gamma}$ (h)	10.7	6.8-13.4
Cl (L/h*kg)	0.44	0.38-0.6
AUC_0^{inf} (ng*h/ml)	113.5	83.0-131.7
Vd (L/kg)	2.1	1.6-3.1

Glucocorticoids have proven to be beneficial in a variety of conditions in the equine patient. Through extensive research the metabolic and anti-inflammatory properties and the mechanisms through which they work have been well documented. Although limited information regarding the pharmacokinetic properties of glucocorticoids is available, increased investigation has been performed evaluating their pharmacodynamic properties. However, further research needs to be performed to evaluate the anti-inflammatory effects of parenteral glucocorticoids in the equine host.

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CHAPTER 2 – Pharmacokinetics and pharmacodynamics of oral dexamethasone in healthy horses

Introduction

Therapeutic immune suppression through the administration of steroidal anti-inflammatory medications is necessary for a variety of conditions in equine medicine. Examples of such disease include, but are not limited to, recurrent airway obstruction (heaves), interstitial pneumonia, immune-mediated disease, hypersensitivity reactions, and non-infectious inflammatory conditions such as inflammatory airway disease (IAD). Therapy for these conditions typically involves intravenous systemic glucocorticoid therapy which is then modified to lower dose, orally administered medication. Oral glucocorticoid administration is continued for several days to weeks depending on the condition. Over a course of tapering dose therapy, medication is eventually discontinued. Although oral preparations of glucocorticoids are commercially produced, they may not be readily available, efficacious, or economically feasible. An example involves the powdered formulation of dexamethasone; Azium^{TM} powder, that is frequently difficult to obtain, and therefore a compounded formulation may be utilized. Other preparations available for oral administration include prednisone and prednisolone, which are poorly absorbed or may be cost prohibitive for extended use, respectively. Subsequently, a common practice of therapy among equine clinicians,is the use of oral administration of the injectable formulation of dexamethasone solution. The administration of oral dexamethasone solution is performed off-label with very limited investigation into its pharmacological effects.

Endogenous glucocorticoids enter the blood in a circadian rhythm after being produced by the adrenal cortex.[1,2] It is well understood that glucocorticoids exert their immunosuppressive and anti-inflammatory properties by altering gene expression or gene repression through genomic

pathways of mRNA production by binding to glucocorticoid receptors within the cytoplasm of target cells.[1,3-6] Due to the rapid effects of glucocorticoids, non-genomic pathways have recently been recognized to play an important role in the anti-inflammatory and immunosuppressive effects of glucocorticoids. Non-genomic pathways play an integral role through the binding of glucocorticoids to membrane receptors that instantly stimulate second messengers and electrolyte transfer rather than mRNA production.[5,7] Dexamethasone, an analogue of prednisolone, has a longer duration of activity and is 25 times more potent than endogenous cortisol.[8]

Systemic glucocorticoid administration has been associated with adverse side effects such as hypothalamic-pituitary-adrenal axis suppression, muscle wasting, hyperglycemia, polyuria, polydipsia, immunosuppression, and laminitis.[4,9-12] Adrenocortical dysfunction is monitored through endogenous cortisol response to ACTH administration. Previous studies have investigated the horse's adrenocortical function in response to exogenous glucocorticoids. Cortisol suppression, indicating adrenal suppression, occurs following intravenous, intramuscular, and interestingly following formulations of inhaled glucocorticoids.[13-16] Short-term parenteral administration of dexamethasone, prednisolone sodium succinate, and aerosolized beclomethasone diproprionate has not been associated with adrenocortical dysfunction evidenced by response to ACTH stimulation testing[10,13-15] However, a single dose of prednisolone acetate and triamcinolone has resulted in adrenocortical dysfunction for 14 to 21 days.[13,17-19]

To the authors' knowledge, the pharmacokinetics and pharmacodynamics of oral dexamethasone solution have been incompletely determined in horses. Pharmacokinetic studies for IM/IV dexamethasone and oral glucocorticoid administration in the form of Azium™ powder, prednisone, and prednisolone have all been reported.[10,13,16,20-22] The standard of oral dosing of glucocorticoids has been based on the bioavailability of Azium™ powder and prednisolone which is reported to be approximately 61% for both when administered to horses.[20,22] Multiple studies have shown oral prednisone to have limited efficacy in the horse.[21-24] Nevertheless, practitioners continue

to utilize oral prednisone in select cases due to the perception that prednisone may have less serious side effects than dexamethasone. Previous studies have proven the benefits of aerosolized glucocorticoids and orally administered dexamethasone solution. Pulmonary function studies examining the efficacy of glucocorticoids in horses have been utilized to measure the effects of aerosolized, systemically administered, and orally administered glucocorticoids to relieve signs of recurrent airway obstruction.[14,15,24-28] Cornelisse et al, performed the first study to evaluate the efficacy of oral administration of dexamethasone solution in horses with clinical disease of recurrent airway obstruction.[14] This study showed improved lung function within 6 hours after administration with peak effect 24 hours following oral administration.[14] The pharmacokinetic and pharmacodynamic effects of aerosolized glucocorticoids have been evaluated rather extensively, and compared to the systemic administration of dexamethasone.[11,14,15,24-27] Although anecdotal experience and limited investigation support the use of injectable dexamethasone solution to be administered orally, the pharmacokinetic and pharmacodynamic effects of orally administered dexamethasone solution have not been extensively evaluated.

Not only has oral dexamethasone solution been shown to be effective against inflammatory conditions it also provides an economical and convenient means for the administration of glucocorticoids. Although there is limited investigation into the pharmacological effects of orally administered dexamethasone solution, veterinary clinicians increasingly utilize injectable dexamethasone solution orally. The focus of our study was to determine the pharmacokinetics of oral dexamethasone solution compared to the intravenous administration of dexamethasone and the administration of oral dexamethasone powder in healthy horses. In addition, pharmacodynamic effects were evaluated based upon systemic cortisol response to the different formulations of dexamethasone and route of administration.

Materials and Methods

Animals—Six healthy adult horses were used in the study approved by the Animal Care and Use Committee at Kansas State University. The horses (4 mares and 2 geldings) ranging from 13 to 27 years of age included 5 Quarter Horses and 1 Dutch Warmblood. Body weight of the horses ranged from 385-630 kg. All horses were considered to be healthy based upon physical examination, complete blood count, and serum biochemistry.

All horses were allowed free access to individual runs, except during the experimental period. During the experimental period, all horses were maintained in individual stalls beginning 18 hours prior to drug administration and throughout the duration of sample collection. Throughout the study all horses had access to fresh water at all times. They were fed their typical complete pelleted diet in two equal feedings and grass hay ad libitum, except when food was withheld starting 8 hours before to 3 hours following drug administration for the fasted phase of the study. All horses were accustomed to handling and venipuncture.

Drug Administration—In a randomized crossover block design, each horse received in a random order: 1) dexamethasone solution 0.05 mg/kg IV, 2) dexamethasone solution 0.05 mg/kg PO, and 3) dexamethasone powder 0.05 mg/kg PO all in the fed and fasted states.

Treatment	Fed/Fasted	Route of Administration	Type of Glucorticoid	Placebo Administered
1	Fed	IV	DXM solution	oral molasses
2	Fasted	IV	DXM solution	oral molasses
3	Fed	Orally	DXM solution	IV 0.9% NaCl
4	Fasted	Orally	DXM solution	IV 0.9% NaCl
5	Fed	Orally	DXM powder	IV 0.9% NaCl
6	Fasted	Orally	DXM powder	IV 0.9% NaCl

Table 2.1: Treatment groups for 0.05 mg/kg dose of dexamethasone with fed/fasted state, route of administration, type of dexamethasone, and type of placebo

To monitor the effects of the study on normal circadian rhythm, 5 of the 6 horses served as controls and received no treatment. To further minimize any effects of normal circadian rhythm, studies started at 8:00 am for all treatment groups. Intravenous administration of dexamethasone solution was performed by the same investigator via venipuncture of the left jugular vein. Oral administration

of dexamethasone solution and powder was performed by the same investigator via oral dosing syringe. There was a minimum washout period of 2 weeks between drug treatments. A commercially available injectable formulation of dexamethasone was used for IV and PO solution administration. Due to the inability to obtain Azium^{TM} powder, a compounded formulation of dexamethasone powder from a reputable equine compounding pharmacy was utilized mixed in liquid molasses for the PO powder administration. When oral dexamethasone was administered an IV placebo was also administered consisting of 0.9% NaCl equivalent to a volume of 2 mg/ml dexamethasone at 0.05 mg/kg. When IV dexamethasone was administered oral placebo consisted of liquid molasses equivalent to the calculated volume of oral dexamethasone solution or powder.

Collection of samples and measurement of drug concentrations—Serial blood samples (10ml) were collected via a 14-gauge, 5.25-inch catheter inserted into the right jugular vein. Prior to catheter placement, an area over the right jugular vein was clipped, aseptically prepared with chlorhexidine gluconate 4%, rinsed with 70% isopropyl alcohol, and the skin infiltrated with 2% lidocaine HCL. Serial blood samples for the control group were taken every two hours for 48 hours. For the treatment groups, initial blood samples were collected 24 hours before drug administration, while still at their normal environment outside the hospital. Serial blood samples were then collected before drug administration (time 0), 15, 30, 45 minutes and 1, 2, 4, 8, 12, 24, 36, 48, and 72 hours after drug administration. Blood samples were immediately transferred to sodium heparin tubes and serum red top tubes. Following centrifugation serum and plasma were frozen at -70˚C until analyzed for plasma dexamethasone and serum cortisol concentrations. Plasma dexamethasone concentrations were analyzed via liquid chromatography-mass spectrometry with a minimum level of quantification of 1ng/ml. Serum cortisol concentrations were analyzed with the use of chemiluminescent enzyme immunoassay with a limit of quantification of 5.5-1,380 nmol/L.

Statistical analysis—Comparisons of pharmacokinetic parameters were performed for all treatment groups (intravenous dexamethasone, oral compounded powder, and oral dexamethasone solution

both in the fed and fasted state) via non-parametric Kruskall-Wallis one-way ANOVA. When significant ($P<0.05$) differences were determined pairwise multiple comparison procedures were performed via Dunn's method. Comparisons of pharmacodynamic parameters (cortisol suppression) were performed for all treatment groups using the mixed procedure for repeated measures. When significant ($p<0.05$) differences were determined pairwise multiple comparison procedures were performed via least square means. Student t-test exercised at each time point was utilized to compare mean cortisol concentrations in the fed and fasted state for each treatment group. To evaluate the circadian rhythm of cortisol secretion one-way ANOVA was utilized with Tukey Kramer HSD for multiple comparisons.

Pharmacokinetic analysis—Plasma dexamethasone concentration versus time data for each animal was analyzed after each treatment through the use of a pharmacokinetic computer program* to estimate variables. A noncompartmental model was used to calculate AUC_0^{∞}, clearance, terminal half-life, and volume of distribution steady state for intravenous administration. For oral administration a noncompartmental model was also utilized to calculate maximum plasma concentration, terminal half-life, and AUC in addition to other parameters. The linear trapezoidal rule was utilized to determine AUC from time zero to infinity. Clearance was calculated by the dose divided by AUC. Volume of distribution steady state (Vd_{ss}) was calculated by the equation: Vd_{ss} = Dose * $AUMC/AUC^2$. The terminal half-life ($t_{1/2}$) was calculated by the equation $t_{1/2} = 0.693 / \lambda z$. For the PK-PD modeling, the calculated pharmacokinetic parameters were best described with a two compartmental model for IV administration with bolus input and first-order elimination. A one compartmental model best described the oral administration treatment groups with first-order input and output with no lag time. After IV administration, plasma concentration (C) of dexamethasone was described by the equation: $C(t) = A^{-\alpha t} + B^{-\beta t}$ where t is time after drug administration; A and B are the y-axis intercepts for the distribution and elimination phases of the curve, respectively, and α and β are the slopes for the distribution and elimination phase of the curve, respectively. Plasma

concentration of dexamethasone after oral administration was described by the equation: $C(t) = D * K_{01}/V(K_{01}-K_{10}) * (e^{-K_{10}*t}-e^{-K_{01}*t})$ where D is the dose of dexamethasone that the horse received, K_{01} is the rate constant for absorption, V is the volume of distribution for the central compartment, and K_{10} is the rate constant for elimination. The fraction of the dose absorbed (%F) of dexamethasone solution and compounded powder after oral administration was calculated by the equation: $\%F = 100\% * AUC_{PO}/AUC_{IV}$ where AUC_{PO} is the AUC following oral administration and AUC_{IV} following IV administration. The time to maximum plasma concentration (Tmax) and maximum plasma concentration (Cmax) of dexamethasone following oral administration were determined by actual measured data points.

Pharmacodynamic analysis—Adrenal cortical suppression was analyzed via serum cortisol response to the methods of dexamethasone administration and formulation of dexamethasone. Actual cortisol response was analyzed by actual measured data points. Predicted pharmacodynamic parameters were estimated by a pharmacokinetic-pharmacodynamic model using a sigmoid inhibitory effect model with a baseline effect parameter. The equation for the sigmoid inhibitory effect model used was: $E = E_{max} - (E_{max}-E_0)[C^{\gamma}/(C^{\gamma}+EC^{\gamma}_{50})]$ where Emax is the maximum suppression of cortisol, E_0 is the cortisol concentration at time zero, C is the concentration of dexamethasone, γ is the shape parameter, and EC50 is the concentration of dexamethasone to produce 50% reduction of cortisol. Predicted pharmacodynamic parameters analyzed included: E_{max}, ECe_{50}, E_0, and rate of drug loss from the effect compartment (Ke0).

Results--Dexamethasone was tolerated well by all horses after both oral and IV administration. No adverse effects were detected.

Mean plasma dexamethasone concentrations over time following IV and oral administration of the different dexamethasone formulations were plotted (Figure 2.1 and 2.2) for both the fed and fasted states. Plasma dexamethasone concentrations were below level of quantification (LOQ=1 ng/mL) 12 hours following dexamethasone administration for all treatment groups except for the

fasted, oral solution which was below the LOQ at 8 hours post-administration. Estimates of the pharmacokinetic and pharmacodynamic parameters for dexamethasone and cortisol for all treatment groups are shown in tables 2.1 and 2.2. At time zero (C0) the estimated plasma dexamethasone concentration following IV administration was 73.92 and 66.34 ng/ml for the fed and fasted states, respectively. Following oral administration, there was a significant difference in maximum measured plasma concentration of dexamethasone (Cmax) between the orally administered dexamethasone solution for both the fed and fasted (9.75 and 9.65 ng/ml) states compared to the compounded powder (23.18 ng/ml) in the fasted state. However, there was no significant difference in the time (Tmax) which Cmax was achieved (0.79 hr – 1.50 hr). Following IV administration, there was a significant difference between $t_{1/2}$ for the fed and fasted state, 2.53 and 3.45 hours, respectively. There was no significant difference between $t_{1/2}$ for the oral dexamethasone solution or powder. For the horses receiving dexamethasone administered by the IV route, the clearance was 7.31 and 7.18 ml/min/kg for fed and fasted treatment groups, the Vdss was 1.27 and 1.69 L/kg for fed and fasted IV treatment groups, and the AUC_0^{inf} was 116.50 and 120.36 hr*ng/mL for fed and fasted IV treatment groups. The AUC_0^{inf} for the orally administered dexamethasone solution was 43.28 and 54.04 hr*ng/mL for the fed and fasted treatment groups. There was a significant difference between AUC_0^{inf} for the compounded powder treatment group of 41.19 and 79.47 for the fed and fasted groups, respectively. The bioavailability for the orally administered dexamethasone solution was 35% and 44% for the fed and fasted treatment groups, respectively. For the orally administered compounded powder treatment group the bioavailability was 33% and 70% for the fed and fasted treatment group, respectively.

Endogenous cortisol versus time plot for dexamethasone administration for all treatment groups is shown by figures 2.3 and 2.4. There was no significant difference between cortisol suppression and treatment. However, there was significant suppression in serum cortisol concentration from baseline concentration starting at 1 hour post-administration for all fasted

treatment groups that continued until 72 hours post-administration. Likewise, there was significant suppression of serum cortisol concentration from baseline for all fed treatment groups starting at 2 hours post-administration and continuing to 48 hours post-administration.

Predicted pharmacodynamic parameters are shown in Table 3. Modeling of the intravenously administered dexamethasone was best described by a two-compartment model with elimination from central compartment, as compared to one-compartment modeling for the orally administered treatment groups. There was no significant difference between the different treatment groups and Emax, E_0, and KEO. However, the difference for EC_{50} between oral dexamethasone solution in the fed state (0.28 ng/ml) was significantly different from the EC_{50} of the IV fasted (0.95 ng/ml) and IV fed (0.93 ng/ml) treatment groups. Predicted cortisol response based upon the pharmacokinetic/pharmacodynamic model are represented in figures 2.5 and 2.6.

Evaluation of endogenous cortisol for the presence of circadian rhythm revealed that there was diurnal variation. Cortisol concentrations at 6:00 am and 8:00 am were significantly higher than those at 8:00 pm and 10:00 pm. Similarly cortisol concentrations at 12:00 pm were significantly higher than cortisol concentrations at 10:00pm. These results are represented in figure 2.7.

		Fasted			Fed		
			Percentiles			Percentiles	
Parameter	**Units**	**Mean**	**25th**	**75th**	**Mean**	**25th**	**75th**
λ_z	**hr**	0.21[a]	0.19	0.22	0.28[a]	0.25	0.30
T ½ λ_z	**hr**	3.45[a]	3.17	3.67	2.53[a]	2.31	2.78
C0	**ng/mL**	66.34	52.88	86.30	73.92	59.89	87.30
AUC_{all}	**hr*ng/mL**	107.29	91.15	130.88	110.02	87.28	125.09
AUC_0^{inf}	**hr*ng/mL**	120.36	101.02	140.15	116.50	95.30	130.67
AUC extrap.	**%**	10.71	6.62	9.04	5.70	4.27	6.56
Vd_{area}	**L/kg**	2.10[a]	1.89	2.39	1.59[a]	1.54	1.68
Vd_{ss}	**L/kg**	1.69	1.40	2.01	1.27	2.66	3.23
Cl	**mL/min/kg**	7.18	5.95	8.25	7.31	6.38	8.74
$AUMC_{all}$	**hr*hr*ng/mL**	295.57	220.59	347.59	255.41	1.55	1.68
$AUMC_{inf}$	**hr*hr*ng/mL**	494.62	331.88	690.04	341.33	180.26	315.62
MRT_{all}	**hr**	2.69	2.42	3.15	2.30	254.64	398.31
MRT_{inf}	**hr**	4.02[a]	3.29	4.40	2.92[a]	1.85	2.67
Superscript letters indicated significant differences (P<0.05) within a row.							

Table 2.1: Noncompartmental pharmacokinetic analysis of IV dexamethasone 0.05 mg/kg to 6 healthy horses

		PO Fast Solution			PO Fed Solution			PO Fast Compounded			PO Fed Compounded		
			Percentiles			Percentiles			Percentiles			Percentiles	
		Mean	25th	75th	Mean	25th	75th	Mean	25th	75th	Mean	25th	75th
Parameter	**Units**												
λ_z	**hr**	0.21	0.16	0.28	0.32	0.28	0.45	0.27	0.26	0.29	0.31	0.19	0.31
T ½ λ_z	**hr**	4.08	2.46	4.83	2.51	1.53	2.46	2.59	2.40	2.63	2.79	2.24	3.68
T_{MAX}	**hr**	1.50	1.00	2.00	1.08	0.75	1.00	0.79	0.75	0.75	1.38	0.75	2.00
C_{MAX}	**ng/mL**	9.75[a]	8.67	12.79	9.65[b]	7.09	14.28	23.18[a,b]	16.75	23.81	11.11	5.50	15.78
AUC_{all}	**hr*ng/mL**	44.75	34.10	55.93	37.85	25.24	51.38	73.04[a]	50.49	82.91	34.50[a]	19.75	48.43
AUC_0^{inf}	**hr*ng/mL**	54.04	41.98	68.51	43.28	29.06	54.34	79.47[a]	58.03	90.68	41.19[a]	27.86	53.08
AUC extrap.	**%**	16.28	11.65	22.32	13.79	9.56	20.50	8.91	4.81	11.86	18.63	9.12	29.12
Vd_{area} / F	**L/kg**	5.85	3.38	7.72	4.61	2.60	6.37	2.52	2.41	3.06	6.41	3.12	9.52
Cl / F	**mL/min/kg**	16.48	12.20	19.91	23.44	15.34	28.68	11.45[a]	9.19	14.36	24.10[a]	15.70	29.91
$AUMC_{all}$	**hr*hr*ng/mL**	235.79	103.68	243.89	119.26	84.76	161.70	224.69	138.20	312.27	98.57	65.42	128.18
$AUMC_{inf}$	**hr*hr*ng/mL**	296.67	206.58	397.13	190.53	127.44	230.98	311.70	225.34	440.62	181.08	173.34	179.50
MRT_{all}	**hr**	5.28	2.97	5.62	3.03	2.79	3.36	2.98	2.72	3.20	3.26	2.50	3.31
MRT_{inf}	**hr**	5.49	4.17	6.47	4.26	3.38	4.39	3.88	3.66	3.88	5.02	3.27	6.22
F	**%**	44	37	54	35	20	43	70	53	89	33	17	40
Superscript letters indicate significant differences (P<0.05) within a row.													

Table 2.2: Noncompartmental pharmacokinetic parameters of dexamethasone, 0.05 mg/kg PO, as the commercially available solution for injection and a compounded formula.

		IV Fasted (n=6)			IV Fed (n=6)			PO Fast Solution (n=5)			PO Fed Solution (n=5)			PO Fast Compounded (n=5)			PO Fed Compounded (n=2)		
			Percentiles			Percentiles			Percentiles			Percentiles			Percentiles			Percentiles	
Parameter	**Units**	**Mean**	**25th**	**75th**	**Mean**	**25th**	**75th**	**Mean**	**25th**	**75th**	**Mean**	**25th**	**75th**	**Mean**	**25th**	**75th**	**Mean**	**25th**	**75th**
E_0	**nmol/mL**	117.78	86.24	156.47	116.90	102.46	126.37	111.88	89.27	134.10	119.60	103.68	143.48	119.80	97.62	140.93	94.70	84.03	105.36
ECe_{50}	**ng/mL**	0.95[a]	0.69	1.14	0.93[b]	0.75	0.98	0.91	0.33	1.16	0.28[a,b]	0.18	0.40	0.52	0.38	0.62	0.44	0.41	0.48
E_{max}	**nmol/mL**	0.66	-10.00	8.60	4.72	0.04	4.10	1.70	-2.83	9.14	-2.47	-8.75	2.49	1.14	-9.58	9.58	2.83	-3.03	8.68
γ		4.41	3.35	4.73	7.66	4.66	7.70	3.88	2.30	5.24	3.63	2.18	5.68	5.04	2.15	9.07	6.16	2.60	9.73
KEO	**1/hr**	0.02	0.01	0.02	0.02	0.01	0.02	0.07	0.02	0.09	0.03	0.02	0.05	0.02	0.01	0.02	0.03	0.03	0.03
R		0.98	0.98	0.98	0.94	0.88	0.97	0.88	0.84	0.96	0.96	0.93	0.98	0.98	0.97	0.98	0.94	0.94	0.94
V1	**L/kg**	0.74	0.56	0.90	0.68	0.49	0.92	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
V/F	**L/kg**	N/A	N/A	N/A	N/A	N/A	N/A	4.03[a]	3.44	4.66	5.55	3.58	7.26	2.17[a]	1.84	2.49	3.68	2.70	4.67
T_{LAG}	**hr**	N/A	N/A	N/A	N/A	N/A	N/A	0.49	0.40	0.65	0.29	0.19	0.41	0.28	0.21	0.41	0.24	0.00	0.49
K_{01}	**1/hr**	N/A	N/A	N/A	N/A	N/A	N/A	5.93	1.86	12.06	4.41	2.11	6.16	5.26	4.41	5.86	19.26	3.30	35.23
K_{10}	**1/hr**	0.60	0.45	0.87	0.79	0.47	1.08	0.27	0.25	0.30	0.28	0.17	0.32	0.35	0.30	0.39	0.31	0.23	0.39
V1	**L/kg**	0.74	0.56	0.90	0.68	0.49	0.92	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
K_{12}	**1/hr**	1.13	0.86	1.33	1.68	0.55	1.93	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
K_{21}	**1/hr**	0.71	0.72	0.83	1.09	0.73	1.87	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
R		1.00	1.00	1.00	0.99	0.99	1.00	0.97	0.96	0.99	0.92	0.88	0.99	0.98	0.96	0.98	0.88	0.77	0.98
Superscript letters indicate significant differences (P<0.05) within a row.																			

Table 2.3: Predicted pharmacodynamic/pharmacokinetic parameters for 0.05 mg/kg administration of IV dexamethasone solution, oral dexamethasone solution, and dexamethasone powder in fed and fasted states

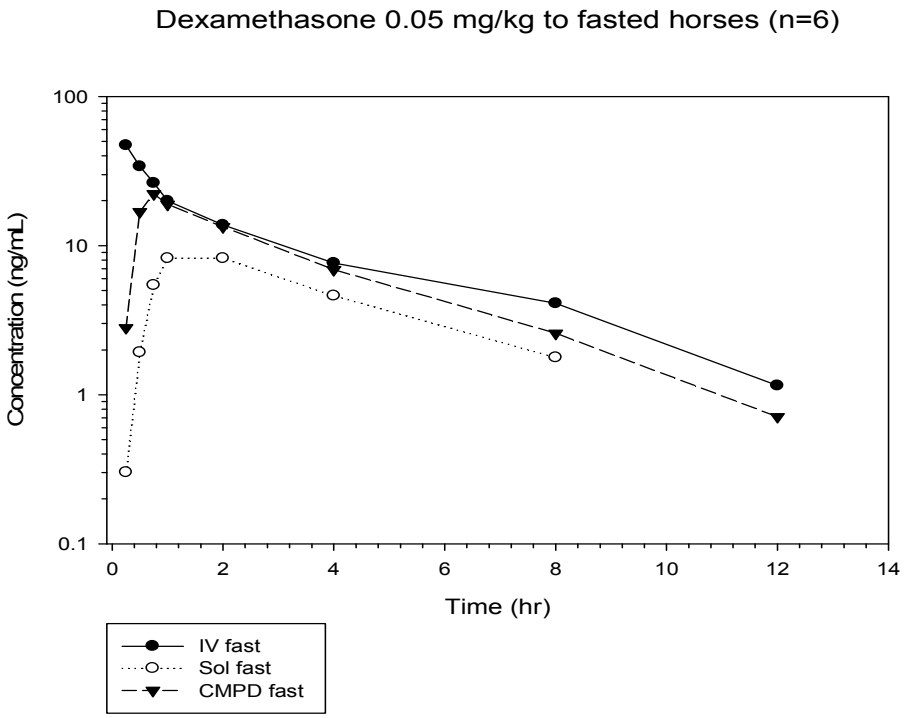

Figure 2.1: mean plasma dexamethasone concentration following 0.05 mg/kg IV and PO administration in the fasted state

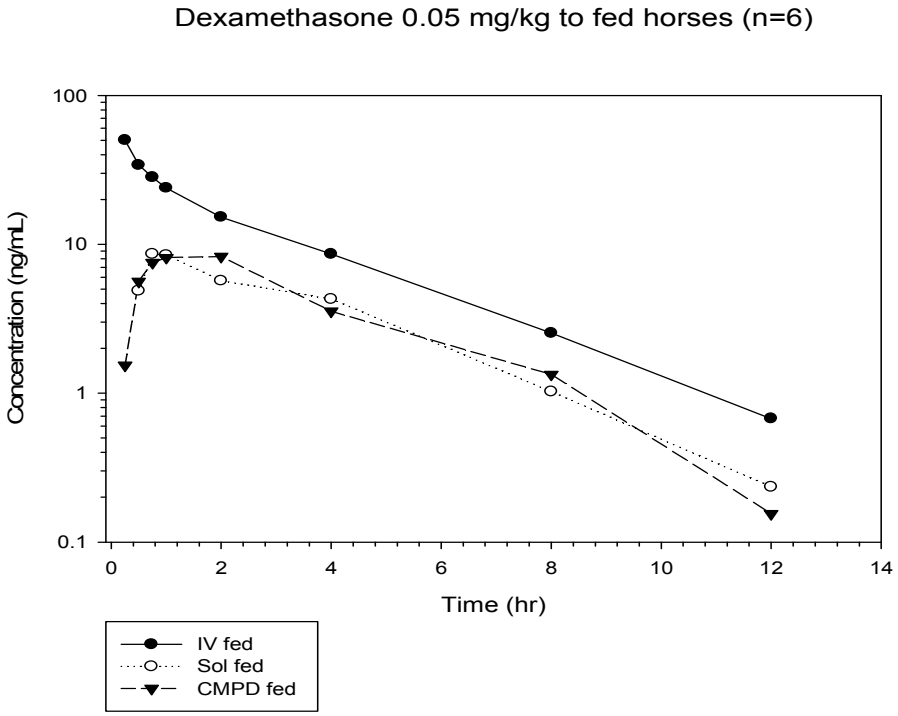

Figure 2.2: mean plasma dexamethasone concentration following 0.05 mg/kg IV and PO administration in the fed state

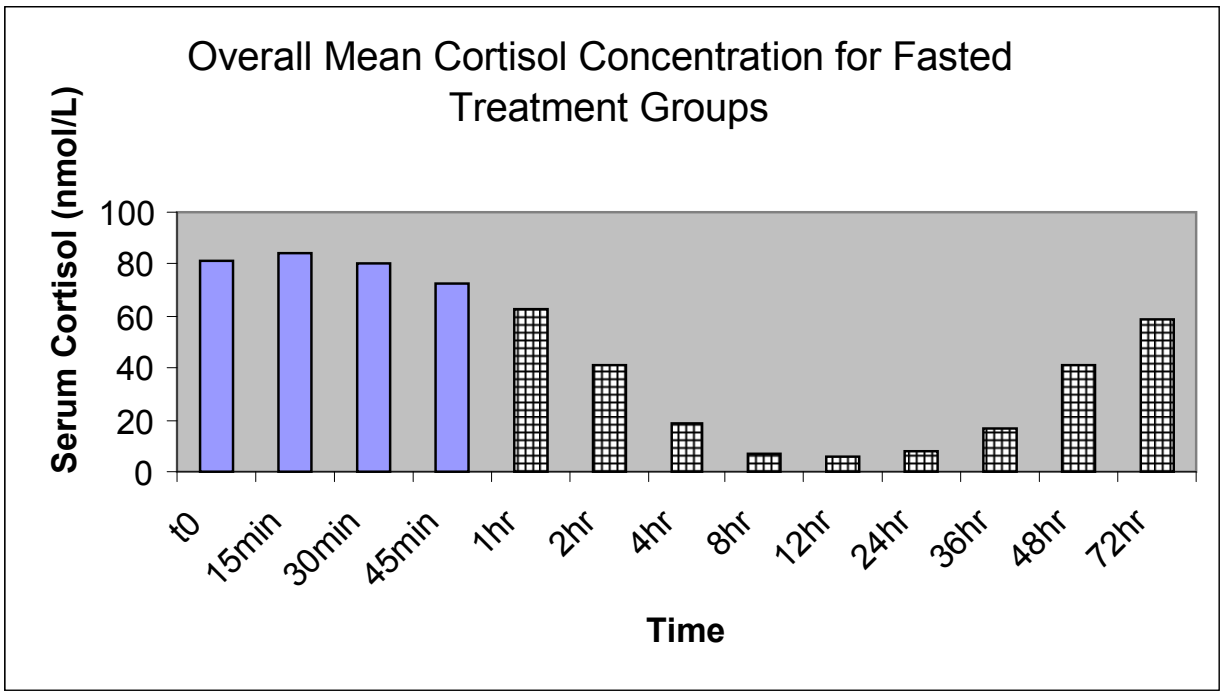

Figure 2.3: Overall mean cortisol suppression for all fasted treatment groups following 0.05 mg/kg dose of dexamethasone (dashed bars significant from baseline: $p<0.05$)

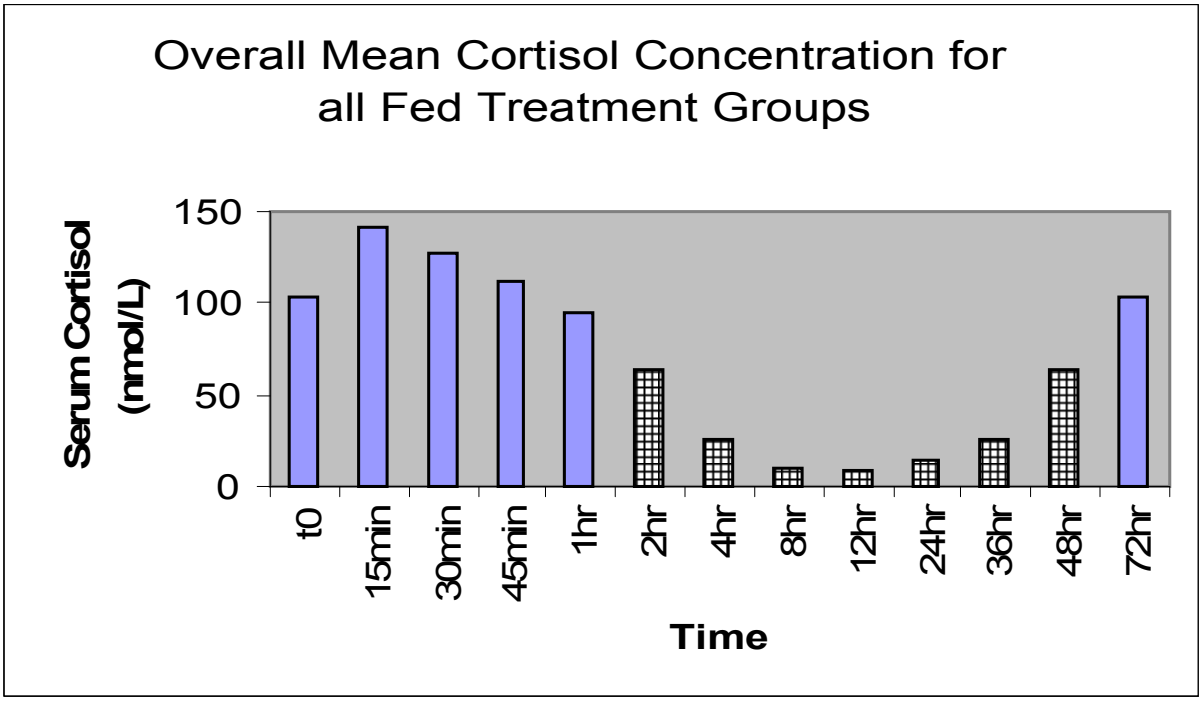

Figure 2.4: Overall cortisol suppression for all fed treatment groups following 0.05 mg/kg dose of dexamethasone (dashed bars significant from baseline: $p<0.05$)

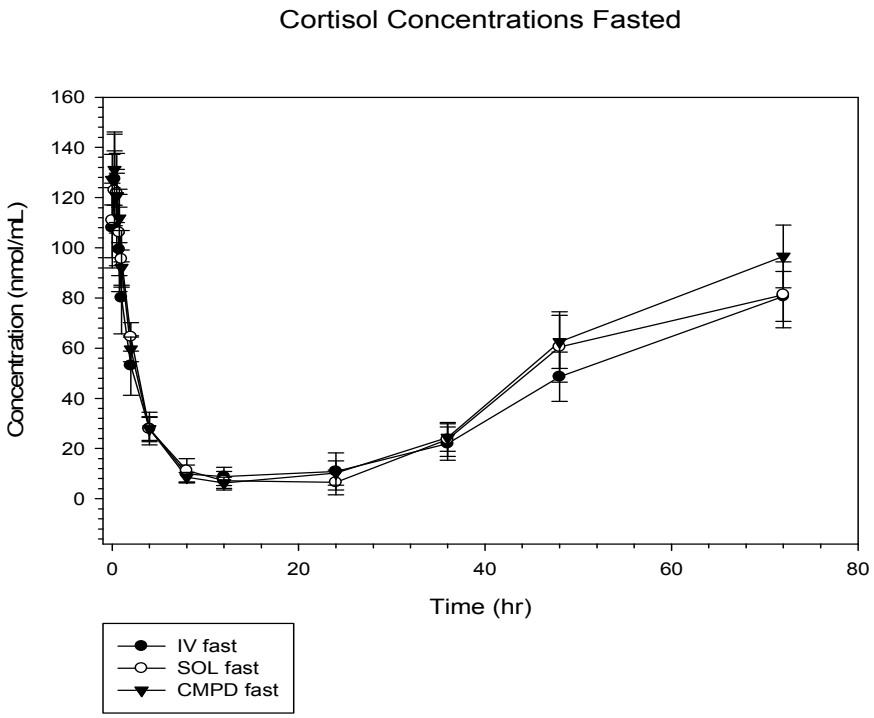

Figure 2.5: Predicted cortisol response based upon pharmacokinetic/pharmacodynamic modeling following 0.05mg/kg dose of dexamethasone

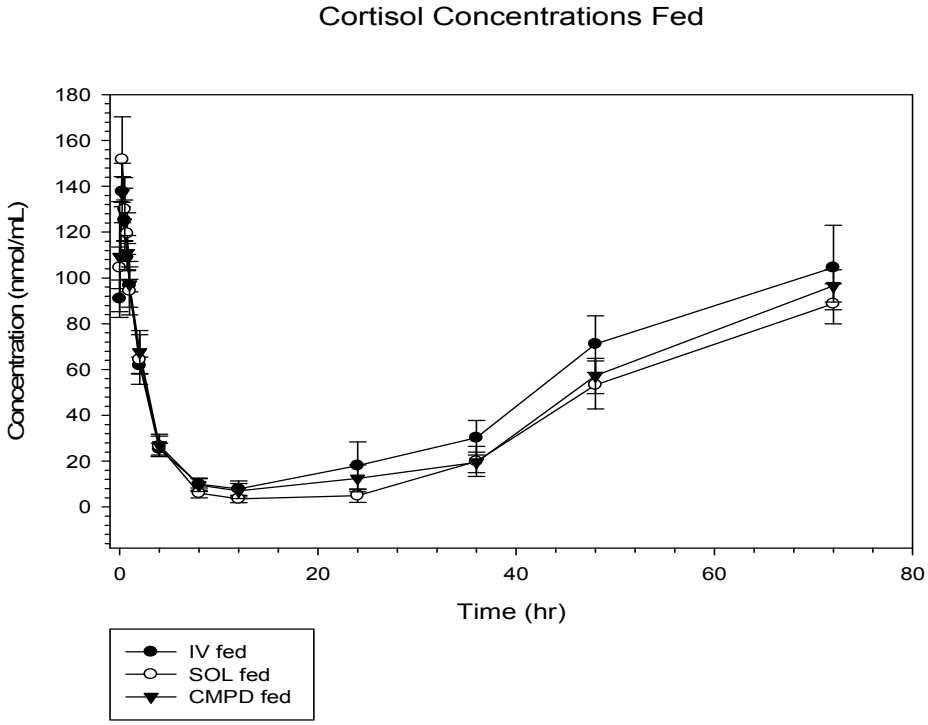

Figure 2.6: Predicted cortisol response based upon pharmacokinetic/pharmacodynamic modeling Following 0.05 mg/kg dose of dexamethasone

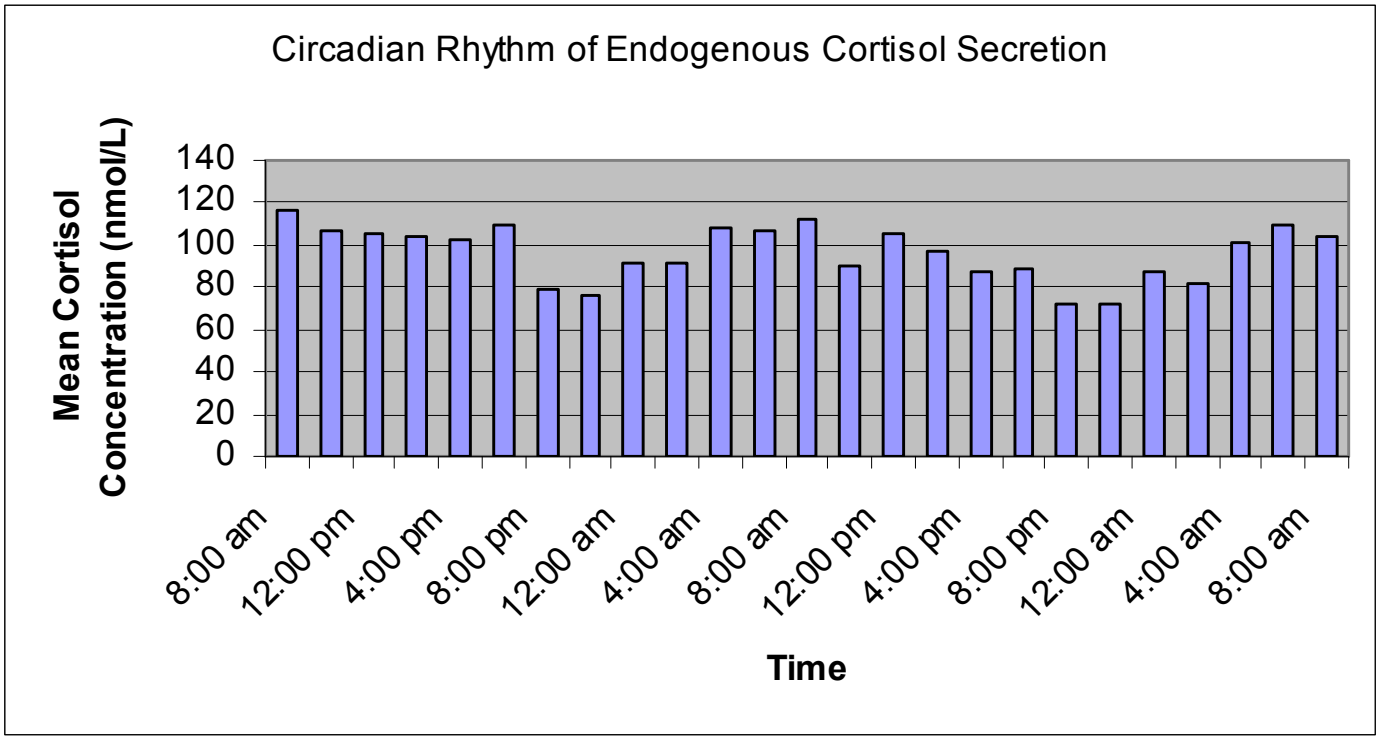

Figure 2.7: Mean circadian rhythm of endogenous cortisol secretion

Discussion— Veterinarians are increasingly utilizing dexamethasone solution orally with limited information regarding its pharmacokinetic and pharmacodynamic properties. This study was designed to determine the pharmacokinetics of oral dexamethasone in horses after receiving dexamethasone solution intravenously and orally, in addition to oral dexamethasone powder. Furthermore, pharmacodynamic properties were evaluated by assessing adrenal suppression through changes in endogenous cortisol concentrations. The effect of feeding on the absorption of orally administered dexamethasone was evaluated by administering all treatments to all horses in both the fed and fasted state.

This present study did not assess adrenal gland function prior to commencing the study due to the apparent health of the horses, and lack of apparent clinical signs associated with pituitary pars intermedia dysfunction. However, when analyzing serum cortisol concentrations one of the horses in the study failed to suppress to levels of the other horses, and had consistently higher cortisol concentrations than the rest of the group. This particular horse was later euthanized and diagnosed with pituitary pars intermedia dysfunction. For completeness of the study, the plasma dexamethasone and serum cortisol concentrations were still factored into the pharmacokinetic and pharmacodynamic parameters.

The circadian rhythm of plasma glucocorticoid concentrations has been documented in humans, rhesus monkeys, rats, dogs, mice, channel catfish, and swine.[2,8,30,31] Some studies performed in the horse have been successful in identifying a circadian rhythm while others have not.[2,31-34] In those studies that have not identified a consistent rhythm it appears that sampling method and subsequent stress on untrained horses has been associated with the lack of a circadian rhythm.[2,34] In the studies that identified a circadian rhythm peak concentrations occurred between 6:00 am and 9:00 am, however trough concentrations occurred between 4:00 pm and 11:00pm.[2,31-33] Much like previous studies, the horses in the present study maintained a circadian rhythm of cortisol

secretion during hospitalization. However, it is important to recognize that they were trained to handling, venipuncture, and hospitalization. We conclude that a circadian rhythm of cortisol secretion exists in horses with a peak in the morning and a trough in the evening, but is fragile and may easily be disrupted when placed in stressful situations. The described investigation did not disrupt the natural rhythm in this group of horses.

Until recently, pharmacokinetic and pharmacodynamic analysis of dexamethasone in the horse has been considered to be incomplete because of the lack of sensitivity of previous analytical methods. However, in 2005, Soma et al., utilized liquid chromatography interfaced with triple spray quadruple quantum tandem mass spectrometry with a sensitivity of 100 pg/ml to evaluate the pharmacokinetic and pharmacodynamic effects of intravenous dexamethasone.[16] Previous studies utilized less sensitive high pressure liquid chromatography or radioimmunoassays to determine the pharmacokinetics of dexamethasone.[13,20] The study by Soma et al., was integral in providing complete pharmacokinetic parameters following IV administration of dexamethasone.[16] In comparison to previous studies which evaluated the pharmacokinetic parameters of IV dexamethasone, the pharmacokinetic results from our study (clearance, volume of distribution, mean residence time, AUC, C0) are similar to those found by Soma et al, as well as those found in the study by Cunningham et al.[16,20] Therefore, the use of liquid chromatography-mass spectrometry with a minimum level of quantification of 1 ng/ml allowed us to accurately characterize the pharmacokinetic nature of dexamethasone.

Our study is the first to evaluate the pharmacokinetics of oral dexamethasone solution in the horse. Cunningham et al, provided the first oral pharmacokinetic study when they evaluated the pharmacokinetics of Azium^{TM} powder.[20] Peroni et al, then performed pharmacokinetic analysis of oral prednisolone several years later.[22] These two studies have served as the benchmark for oral dosing of glucocorticoids with bioavailabilities of approximately 61% for both the Azium^{TM} powder and prednisolone.[20,22] The Tmax and the elimination $t_{½}$ reported by Cunningham et al, (1.3 ± 0.5 hr

and 4.36 ± 1.34 hr) was similar to those in the present study for all oral treatment groups (ranging from 0.79 hr – 1.5 hr and 2.5 hr – 4.08 hr).[20] For all oral dexamethasone treatment groups, the Cmax achieved in our study ranged from 9.75 ng/ml to 23.18 ng/ml as compared to 4.9 ng/ml for the Azium™ powder reported by Cunningham et al.[20] Oral dexamethasone formulations in this study resulted in greater AUC_0^{inf} (41.19 hr*ng/ml – 79.47 hr*ng/ml) compared to the results found by Cunningham et al, (29.09 ± 8.69 hr*ng/ml).[20] Bioavailability in both studies was extremely variable ranging from 33% for the fed, compounded powder to 70% for the fasted, compounded powder in the present study. Bioavailability for oral dexamethasone solution was 44% for the fasted solution and 35% for the fed solution. Although there was no significant difference in bioavailability, the fasted compounded powder reached significantly higher maximum serum concentrations and had a much higher bioavailability of 70%. The lack of significance in bioavailablility may be due to the extreme variability in oral absorption, and the limitations on the number of horses in the study.

In the present study and previous studies, significant adrenal suppression as evidenced by suppression of cortisol concentrations from base-line was recognized starting 1-2 hours post administration of IV dexamtethasone.[13,16] This suppression was preceded by slight increase in cortisol concentration at the first measured time point following dexamethasone administration.[13,16] It is possible that this could be associated with the administration of the medication, or it has been proposed that this may be associated with normal intra-circadian fluctuations with intermittent peaks and troughs.[2,16,33] Horses in our study were catheterized and trained to handling and venipuncture, and care was taken to minimize excitement during administration of dexamethasone and during sample collection. Cortisol suppression in the present study was similar to cortisol suppression found by Soma et al.[16] For all the fasted treatment groups in our study, cortisol suppression was significant starting 1 hour following administration and remained significantly suppressed until 72 hours post-administration which followed the same pattern found by Soma et al.[16] In the fed treatment groups, cortisol suppression was significant starting at 2 hours until 48 hours. Despite the fact that plasma

dexamethasone concentrations were not detected beyond 12 hours in our treatment groups, cortisol remained suppressed for 48 to 72 hours. These findings in conjunction with the relatively high volume of distribution supports the idea that concentration at the cellular level is more important than plasma concentration.[13,16] In addition, changes in transcription and translation may lag behind changes in drug concentration whether it is in the plasma, or at the glucocorticoid receptor.

Because of the increased sensitivity of the analytical methods utilized by Soma et al, they were able to identify a third compartment that no other studies have identified.[16] Due to the limit of quantification of the liquid chromatography-mass spectrometry used in the current study a two compartment model for the pharmacokinetic/pharmacodynamic modeling was identified. It is speculated that the third compartment maintains effective dexamethasone concentrations and subsequently is responsible for the delayed recovery of serum cortisol concentrations.[16] However, it is well understood that glucocorticoids exert their immunosuppressive and anti-inflammatory properties by altering gene expression or gene repression through genomic pathways of mRNA production by binding to glucocorticoid receptors within the cytoplasm of target cells.[1,3-6] Due to the rapid effects of glucocorticoids, non-genomic pathways have recently been recognized to play an important role in the anti-inflammatory and immunosuppressive effects of glucocorticoids. Non-genomic pathways play an integral role through the binding of glucocorticoids to membrane receptors that instantly stimulate second messengers and electrolyte transfer rather than mRNA production.[5,7] The genomic effects of glucocorticoids generally take hours to days to become evident. It is still possible that the delayed recovery of serum cortisol concentration could be a result of the genomic effects and subsequent gene expression or gene repression.

In the pharmacodynamic studies that have monitored the effects of glucocorticoids on pulmonary function in horses with recurrent airway obstruction some have also measured adrenal suppression through measuring cortisol concentrations.[11,12,29,] In these studies cortisol suppression has corresponded with clinical improvement. Rush et al, administered IV dexamethasone and

aerosolized beclomethasone diproprionate to horses with clinically induced recurrent airway obstruction.[25,29] Significant suppression of cortisol was present at the first measured time point (2 days) following parenteral dexamethasone and aerosolized beclomethasone administration.[11,15,25,29,] Significant improvement in pulmonary function was reported three days (the first measured time point) following glucocorticoid administration.[11,15,25,29] The improved lung function was maintained one day following the last dose of aerosolized beclomethasone and three days following the last dose of IV dexamethsone.[25,29] The pattern of improved lung function paralleled the suppression of endogenous cortisol. Furthermore in the study by Cornelisse et al, IV dexamethasone resulted in significant improvement in lung function within 2 hours and reached a peak effect at 4-6 hours.[14] In fasted horses, they found that oral dexamethasone solution resulted in improved lung function in 6 hours with peak effect in 24 hours.[14] However, it is important to note that a correlation between cortisol suppression and improved lung function is not necessarily a cause and effect or in this case truly a marker of immune effects, but changes were in parallel, suggestive it may be a surrogate marker, further studies are needed.

Conclusion-- The pharmacokinetics measured in this study were similar to those previously reported. However, this was the first study to evaluate the pharmacokinetics of orally administered dexamethasone solution. Our study effectively measured the pharmacokinetics of IV dexamethasone solution, oral dexamethasone solution and dexamethasone powder, and assessed the endogenous cortisol response. Although the fasted, compounded powder achieved higher plasma concentrations and bioavailability, there was greater variability when compared to the oral solution. In addition, cortisol suppression was similar among all treatment groups indicating similar pharmacodynamic response regardless of the plasma concentrations of dexamethasone.

It has been suggested that it is possible to approximate the duration of therapeutic effects of synthetic and natural glucocorticoids by evaluating the hypothalamic pituitary-adrenal suppressive activity, and the degree of adrenal suppression corresponds with the drug's anti-inflammatory

potency and metabolic half-life.[10,13,35] Based on the findings in this study, it is possible that lower dosages or less frequent administration could have the same pharmacodynamic effects. Therefore, future direction should include pharmacodynamic modeling utilizing reduced dosages. The duration of cortisol suppression may also indicate that every other day dosing may be similarly effective as everyday dosing, and minimize the adverse side effects associated with glucocorticoid administration. The pharmacokinetic/pharmacodynamic model described here in combination with other pharmacokinetic/pharmacodynamic models will be very useful in predicting pharmacodynamic effects and developing anti-inflammatory models for determining further dosing regimens.

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