Haptoglobin concentrations in dogs undergoing trilostane treatment for hyperadrenocorticism

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Background: Increased concentrations of haptoglobin (Hp), a moderate acute phase protein, have been demonstrated in dogs with hyperadrenocorticism (HAC). Monitoring serum concentrations of Hp in hyperadrenocorticoid dogs before and after trilostane administration may provide valuable information on the response to therapy. Objective: The aim of this study was to measure Hp concentrations in dogs with spontaneously occurring HAC at the time of diagnosis and after treatment with trilostane. Methods: Serum Hp concentration was measured using an automatic biochemical assay based on Hp-hemoglobin binding and utilizing SB-7 reagent in 12 dogs with spontaneous HAC before and after treatment with trilostane (30 or 60 mg PO q 12–24 h). Post-treatment Hp concentrations were measured at the time the owner reported an improvement in clinical signs. Pretreatment and post-treatment Hp values were compared with reference values and with values from 4 healthy control dogs. Results: Two dogs with HAC had pretreatment Hp values within the reference interval; 10 dogs had moderate (n = 8) or marked (n = 2) increases in Hp concentration. After treatment with trilostane, Hp concentration remained within the reference interval (n = 2), decreased to within the reference interval (n = 3), or remained moderately increased (n = 7; 3–10 g/L). Overall, a significant decrease was observed in Hp concentration after trilostane treatment compared with pretreatment values (P < .005). Both untreated and treated dogs with HAC had significantly higher Hp concentrations (P < .001) when compared with control dogs. Conclusions: Clinical control of HAC did not closely relate to serum Hp concentration. Further studies are required to assess whether this is because of inadequate control of disease or because a build-up of cortisol precursors or secondary effects of HAC affect Hp concentration. (Vet Clin Pathol. 2005;34:255–258)

Key Words: Acute phase protein, dog, haptoglobin, hyperadrenocorticism, trilostane

Acute phase proteins (APPs) are glycoproteins produced by the liver after stimulation by proinflammatory cytokines released by activated granulocytes and macrophages at sites of inflammation. The acute phase response promotes healing and limits tissue damage after trauma, infection, or inflammation. APPs include haptoglobin (Hp), C-reactive protein (CRP), serum amyloid A, and alpha-1-acid glycoprotein. The concentrations of APPs in the bloodstream are related to the severity and type of the underlying disease process. Haptoglobin is considered a “moderate APP” in dogs because its concentration typically increases 2- to 3-fold in response to inflammation, infection, surgery, or trauma. Interleukin-6 is thought to be the main stimulator of Hp synthesis. Previous studies have documented that prednisolone results in an increase in serum haptoglobin concentration and suggest that endogenous steroids may also stimulate Hp synthesis. Dogs with hyperadrenocorticism (HAC) have been shown to have moderately increased concentrations of Hp, but concentrations were lower than those of dogs receiving exogenous glucocorticoids, suggesting that endogenous glucocorticoids may not stimulate Hp to the same degree as exogenous glucocorticoids. This response to glucocorticoids could be related to the induction of steroid-induced alkaline phosphatase, which also occurs as a canine-specific response by hepatocytes to increased glucocorticoid levels.

Trilostane (Modrenal, Arnolds Veterinary Products Ltd, Shrewsbury, UK) is an orally administered competitive inhibitor of 3β-hydroxysteroid dehydrogenase that blocks the production of a number of endogenous steroids, including cortisol. We hypothesized that successful management of HAC with trilostane would result in a decrease in serum Hp concentration. The aim of this study was to measure Hp concentrations in dogs with spontaneously-occurring HAC at the time of diagnosis and after treatment with trilostane. Monitoring Hp concentration in dogs with HAC before and after treatment could provide valuable information on the stage of clinical control and response to therapy.

Materials and Methods

Dogs and study design

Haptoglobin concentrations were measured in serum from dogs with spontaneous HAC that were referred to the medicine department of the University of Glasgow Veterinary School from January 2000 to December 2003. The criteria for inclusion in this prospective study were appropriate clinical...
signs (polyuria, polydipsia, polyphagia, abdominal distension, panting, skin changes, hepatomegaly), biochemical and CBC findings (increased alkaline phosphatase activity, leukocytosis with mild mature neutrophilia), a cortisol concentration of 600 nmol/L 1 hour after intravenous administration of adrenocorticotropic hormone (ACTH) (Synacthen, Alliance Pharmaceuticals Ltd, Wiltshire, UK) or failure of 0.015 mg/kg dexamethasone to suppress cortisol levels 8 hours after administration, and evidence of unilateral or bilateral adrenal gland enlargement on abdominal ultrasound. Dogs weighing <5 kg received 250 μg ACTH, whereas dogs weighing <5 kg received 125 μg. The 12 dogs with spontaneous HAC that were included in the study ranged from 5 to 12 years in age and included 8 purebred and 2 crossbred dogs.

Samples were also obtained from 4 healthy control dogs that were being evaluated in our hospital as part of routine preanaesthetic assessment. The control dogs were all Greyhounds and ranged in age from 3 to 4 years. Dogs in the control group had no clinical or biochemical evidence of inflammatory disease based on physical examination and serum biochemical and CBC results.

Haptoglobin concentration was measured in serum samples from the dogs with newly diagnosed HAC, prior to ACTH administration, and again after treatment with trilostane (30 or 60 mg PO q 12–24 h). Post-treatment Hp concentrations were measured at the time the owner reported an improvement in clinical signs (decreased polyuria, polydipsia, and polyphagia). An ACTH stimulation test was performed concurrently to assess clinical response to trilostane administration. Good control of HAC was defined as a cortisol value of <150 nmol/L at 4–6 hours post-ACTH.

Haptoglobin measurement

Haptoglobin concentrations were measured with an automatic biochemical assay based on Hp-hemoglobin binding and utilizing SB-7 reagent (Tridelta Development Ltd, County Wicklow, Ireland). Precision of the assay was previously assessed by calculation of intra- and interassay coefficients of variation (CVs). Intra-assay CV, based on duplicate determinations on 40 samples, was 4.3% over a Hp concentration of 0–1.7 g/L. Interassay CV, based on assay of control samples included with each of 12 assays was 4.1% with a Hp concentration (mean ± SD) of 0.24 ± 0.01 g/L, and 8.9% with a Hp concentration of 1.04 ± 0.07 g/L. Accuracy was confirmed in a previous study with parallel dilutions between standards and serum from dogs with increased Hp concentration and also by significant correlation to Hp concentration determined by immunoassay. The reference interval for canine Hp in our laboratory was previously reported as 0–3 g/L. This reference interval concurred with the findings of a previous study by Eckersall et al. Based on this interval, the magnitude of increase in Hp concentration was defined as moderate (3.0–10.0 g/L), or marked (> 10 g/L).

Statistical analysis

Student’s t-tests (Excel 2000, Microsoft Corp, Redmond WA, USA) were used to compare paired data in dogs with HAC. Two-sample t-tests assuming unequal variances were used to compare groups. Significance was set at P < .05.

Results

All control dogs had Hp concentrations within the reference interval. Two dogs (nos 5 and 6) with HAC had pre-treatment Hp concentrations within the reference interval; the remainder had either moderate (n = 8) or marked (n = 2) increases in Hp concentration (Figure 1, Table 1).

After treatment with trilostane, 8 of 12 dogs had post-ACTH cortisol concentrations of <150 nmol/L (5 dogs had
post-ACTH cortisol <70 nmol/L), which was consistent with
good clinical control. Four dogs had improved clinical signs,
but post-ACTH cortisol levels were 150–390 nmol/L, suggest-
ing poor control. Post-treatment Hp levels were within the
reference interval in 5 dogs (nos 4, 5, 6, 7, and 11) (Figure 1).
Three of the 5 dogs had post-ACTH cortisol concentrations
consistent with good clinical control. The remaining 7 dogs,
5 of which were well controlled, continued to have mild to
moderately increased Hp concentrations (3–10 g/L) post-
treatment, although Hp levels were decreased compared with
pretreatment levels. In 2 well-controlled dogs (nos 3 and 5),
Hp concentration increased slightly after trilostane therapy. In
dog 5, although Hp concentration increased after trilostane
administration, both pre-and post-treatment values were
within the reference interval.

Comparison of pretreatment and post-treatment values
showed a significant difference (P < .005) in Hp levels, with
lower concentrations post-treatment (Table 1). Both pretreat-
ment and post-treatment Hp levels were significantly higher
when compared with the control group (P < .001).

Discussion

To our knowledge, this is the first report of Hp concentrations
in dogs with HAC receiving trilostane. Although the number
of dogs evaluated was small, and the results should be
interpreted with caution, the data suggest that although mean
values decrease after treatment, Hp concentration does not
return to reference values in many dogs, such that factors
other than cortisol may be contributing to Hp synthesis in
HAC. Although the number of control dogs was low, Hp
concentrations in the control group in this study confirmed
values obtained previously in our laboratory for healthy
dogs.10 Haptoglobin concentrations in healthy Greyhounds
also compare favorably with Hp levels in healthy dogs of
other breeds.11 In the United Kingdom, strict guidelines
prevent the use of animals in experimental procedures;
therefore, it is difficult to obtain samples from healthy animals
to use as a control group.

Exogenous corticosteroid administration with predniso-
one has been shown to increase Hp concentration. Harvey
and West8 hypothesised that Hp synthesis may occur after
stimulation by endogenous cortisol. In their study, adminis-
tration of prednisolone twice daily for 3 consecutive days
resulted in increased concentrations of Hp that were still
increased by day 9. A more recent study reported that dogs
with HAC and high concentrations of endogenous cortisol
had increased concentrations of Hp, but levels were lower
than in dogs receiving glucocorticoids.5 It was therefore
hypothesized that endogenous corticosteroids do not stimu-
late Hp to the same degree as exogenous prednisolone. In
a recent study in dogs, Hp concentration remained increased
for 2–3 weeks after continuous administration of exogenous
prednisolone.13 Marked increases in Hp (>10 g/L) were found
in that study after administration of 2.2 mg/kg prednisolone
PO q 24 hr for 7 days. More moderate elevations in Hp were
associated with administration of lower doses of prednisolone
(1 mg/kg q 34 hr) over a similar period of time.

In this study, Hp concentrations were increased in the
majority of dogs with untreated spontaneous HAC. Two dogs
with untreated HAC, however, had values within the
reference interval. The reason for this was not clear. Both
dogs had strong physical and clinicopathologic evidence to
support a diagnosis of HAC. Animals with HAC often have
concurrent diseases (eg, urinary tract infection, skin disease).
A previous study demonstrated that Hp concentration is
increased in dogs with a variety of diseases, including
neoplasia, immune-mediated disease, and diabetes mellitus.5
Many of these diseases are likely to be associated with
inflammation. The acute phase response with resultant
increase in Hp concentration in dogs with HAC could be
attributed to associated inflammation rather than being a
direct effect of endogenous cortisol production. At the time
of diagnosis, most dogs with HAC have mild neutrophilia,
which, even in the absence of a left shift, could indicate chronic
inflammation as well the effect of cortisol. Many dogs with
HAC also have secondary dermatologic changes such as
pyoderma, which could stimulate the acute phase response.
Thus, the increases in Hp concentration in dogs with HAC
may not be mediated by cortisol alone.

Trilostane is a competitive inhibitor of 3β-hydroxysteroid
dehydrogenase, resulting in decreased production of several
adrenal steroids such as cortisol and aldosterone.6,14 Loss of
negative feedback could result in increased endogenous
ACTH and cortisol precursors. It is unknown whether
endogenous ACTH or cortisol precursors can directly stimu-
late Hp synthesis, but this could potentially explain why Hp
concentration increased in 2 dogs after trilostane treatment.
These dogs were both tightly controlled as determined by
post-ACTH cortisol values, and neither dog had clinical
evidence of inflammatory disease, although subclinical
inflammation was possible. Alternatively, perhaps overtight
control of cortisol production has an unexplained effect on Hp
synthesis; however, only 2 of 5 dogs tightly controlled with
trilostane (post-ACTH cortisol concentration <70 nmol/L)
had increased Hp concentrations. Ruckstuhl et al13 suggested
that better control of clinical signs of HAC was achieved when
post-ACTH cortisol concentration was <70 nmol/L because
of the short duration of action of trilostane. After trilostane
therapy, Hp concentrations decreased in the majority of dogs
and were significantly different from pretreatment values.
Even in dogs with Hp concentrations within the reference
interval after therapy, mean values were significantly higher in
post-treatment samples compared with control dogs.

Once-daily administration of trilostane suppresses corti-
sol production for <24 hours in dogs.16 Post-ACTH cortisol
concentrations measured 24 hours after trilostane adminis-
tration are considerably higher than those measured 4–6 hours
post-ACTH.17 It is therefore possible that Hp concentration
remained increased in dogs with clinical evidence of good
control of HAC because of lack of cortisol suppression over the
entire 24-hour period. Conversely, post-ACTH cortisol con-
centration may be suppressed before the clinical effects of
HAC, such as dermatologic changes and vacuolar hepato-
pathy, resolve. These secondary effects may result in increased
levels of endogenous cortisol, which could directly influence
Hp production. Liver aspirates were not performed on dogs in
this study, so resolution of hepatic vacuolar changes could not
be assessed. A recent study by Neiger and others proposed
that administration of exogenous ACTH during stimulation
testing may override the reversible inhibition of cortisol
synthesis by trilostane, such that urine cortisol:creatinine ratio
may be a better indicator of therapeutic efficacy. However,
ACTH stimulation test results remain the best indicator of
control in HAC and therefore were used in this study as the
basis for determining if HAC was adequately controlled.

Further paired studies assessing changes in Hp concentra-
tion in a larger population of dogs with HAC and
sequential sampling at various stages of control are indicated
based on the results of this study. In addition, urine cortisol:creatinine ratio should be used in future studies to
obtain further information regarding stage of control. The
results of this preliminary investigation suggest that Hp
concentration decreases but does not return to reference
values after successful stabilization of HAC with trilostane in
most dogs. Additional studies are required to assess whether
this is because of inadequate control of disease or because
a build-up of cortisol precursors or secondary effects of HAC
affect Hp concentration. Other APPs that are unaffected by
administration of corticosteroids, such as CRP, have yet to
be studied in dogs with HAC. Further studies demonstrating
Hp and CRP concentrations in a larger group of dogs with
HAC at various stages of control are in progress.

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