Adrenal responsiveness in critically ill dogs: prospective study

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SUMMARY

The present study was carried out to evaluate adrenal responsiveness in critically ill dogs. We compared adrenal response among thirty-four critically ill dogs (25 survivors and nine non-survivors), and 32 healthy dogs. Each dog underwent an ACTH-stimulation test using a supra-maximal dose (0.25 mg). First basal cortisol and basal aldosterone concentrations were measured. Cortisol/aldosterone test concentrations following stimulation were then measured for each dog. High basal cortisol concentration discriminated critical illness from healthy state. High basal aldosterone and high cortisol values following ACTH-stimulation pointed out a poor prognosis. We observed an activation of adrenal responsiveness in critically ill dogs. Adrenal response appeared to be enhanced by the severity of the disease. We were unable to evaluate if this response was appropriate for the patient’s status. The evaluation of adrenal response appears to be challenging and unfortunately lacks reliable monitorable endpoints.

Keywords: Critical care - dog - cortisol - aldosterone - adrenal responsiveness.

Introduction

The adrenal function in critically ill veterinary patients is currently poorly documented. The activation of the hypothalamic-pituitary-adrenal (HPA) axis stands as a critical component of the general adaptation to stress and the response to critical illness [1,4,19]. During the acute phase of illness, cytokines, as well as other factors, lead to an activation of HPA axis and a subsequent elevation of blood cortisol concentrations [5,25,30]. However, during the last decade, numerous studies in human medicine have pointed to an inadequate production of cortisol relative to the degree of illness, particularly in septic shock. Cytokines and TNF-alpha in particular reduce CRH mediated ACTH secretion and may decrease the cortisol response to the adrenocorticotropic hormone (ACTH), causing poor adrenal activity; moreover lymphocytes may become resistant to corticosteroid [2,4,12,22,23,24,25]. This inappropriate glucocorticoid response and action is called occult adrenal failure or relative adrenal insufficiency (RAI) [4,17,21,22,31,33]. For these reasons, it has been hypothesized that low dose glucocorticoid administration could be beneficial in septic shock [1,6,7]. While, there is a lot of information in humans about this potential RAI which may occur during septic shock, relatively little is known about the occurrence of RAI in other critical illnesses [3,5,9,13,17,18,27,28].

The only prospective study currently available in veterinary medicine failed to identify the development of true adrenal insufficiency in critically ill dogs during hospitalization in intensive care units [26]. The potential benefit of glucocorticoid administration in critically ill dogs and cats has remained undetermined so far.

Regarding current knowledge in humans and the effect on treatment recommendations as well as the lack of information in dogs, it appears particularly important to know whether adrenal responsiveness might be reduced in critically ill dogs.

The purpose of this study was to evaluate adrenal responsiveness in a population of critically ill dogs in our intensive care unit, and to determine if corticoid supplementation could be beneficial during the phase of critical illness. Adrenal responsiveness was assessed by the measurements of basal cortisol, and serum cortisol / aldosterone concentrations following ACTH-stimulation.

Materials and methods

STUDY DESIGN

This study was approved by the Comité d’Éthique de l’École Nationale Vétérinaire de Lyon. This prospective study used a population of 34 critically ill dogs admitted to the SIAMU® (École Nationale Vétérinaire de Lyon ICU) and a population of 32 healthy dogs.
PATIENT SELECTION

All subjects were admitted to the SIAMU® between May 1, 2003, and May 1, 2004, and were selected on the following criteria: severe disease (life-threatening without aggressive treatment in the following 8 hours), and probability of survival greater than 24 hours. Criteria for exclusion were preexisting known adrenal disease, administration of short acting steroids within the preceding 48 hours, or administration of long acting steroids in the three months prior to admission. Thirty four dogs were included (Table I), sixteen males (47%), 18 females (53%). The average age was 5.0 ± 0.7 years (SEM). In the critically ill dogs there were twenty-five survivors (74%, mean age 4.0 ± 0.7 years), and nine non-survivors (26%, mean age 7.8 ± 1.3 years). The category of disease recorded in the dogs included viral gastroenteritis (8), enterectomy (3), acute renal failure (3), cardiac failure (3), pancreatitis (2), gastric volvulus (2), esophagus surgery (2), head trauma (2), encephalitis (2), pancytopenia (1), sepsis (3), thoracic wound (1), pericardial effusion (1), diabetic ketoacidosis (1).

CONTROL HEALTHY DOGS

Twenty Beagles of the 34 healthy control dogs came from the Physiology Unit (20), and 12 (3 Spaniels, 3 Labradors, 1 Boxer, 1 Burmese, 1 Argentinean, 1 White Shepherd, 1 Mongrel, 1 Border Collie) belonged to veterinary students. The average age was 5.1 ± 0.4 years.

BLOOD PARAMETERS

Plasma sodium and potassium concentrations were measured using an ion-specific electrode system (Opti CCA ; AVL). Cortisol assays were performed using radioimmunoassay (Coat-a-Count ; DPC) specifically validated for use in the dog. The limit of quantification for this method was below 5 nmol/L. Aldosterone assays were performed using the radioimmunoassay method (Aldock-2 assay system; DiaSorin). The limit of quantification for this method was below 55 pmol/L. Delta-cortisol (difference between cortisol following ACTH-stimulation and basal cortisol), and delta-aldosterone (difference between aldosterone following ACTH-stimulation and basal aldosterone) were calculated.

STATISTICAL ANALYSIS

All data were recorded in an electronic database (Microsoft Excel). Statistics were compiled with a specific program (Statview ; SAS Institute). All results are given as mean ± SEM. Comparison between control dogs, survivors and non-survivors was performed using a one-way analysis of variance (ANOVA) followed by a Fisher’s test. The significance threshold was set at 0.05. The concordance correlation coefficient Pearson (p’) was computed for basal cortisol or basal aldosterone, cortisol / aldosterone concentrations following ACTH-stimulation, delta-cortisol and delta-aldosterone.

STUDY PROTOCOL

Within twenty-four hours after admission, at 10 a.m., heparinized blood samples were collected. The heparinized blood was centrifuged and the plasma was then separated. The plasma was kept frozen at -20°C until sodium, potassium, cortisol and aldosterone assays. Systolic blood pressure was measured using the indirect method of Doppler ultrasonography (Sega 811-BL ; Parks Medical Electronics). A mean value was computed with five successive measurements. An ACTH stimulation test was performed as an IV bolus of 0.25 mg of Synactène® (Novartis Pharma SA). A second heparinized blood sample for cortisol and aldosterone determination was collected 45 minutes after the injection. Non-survivors were grouped together for data analysis. Control dogs followed the same protocol and were hospitalized in the intensive care unit with similar conditions of stress as critically ill dogs.

Results

CORTISOL (EXPRESSED IN NMOL/L AND PRESENTED AS MEAN (MIN-MAX)) (Table I, Figure 1)

Survivors [34 (5-259)] and non-survivors [65 (10-186)] had basal cortisol concentrations significantly higher as compared to control dogs [25 (5-105)]. Non-survivors showed higher values as compared to survivors but the difference was not significant.

Cortisol concentrations following ACTH-stimulation were significantly higher in non-survivors [214 (109-602)] than in survivors [187 (80-470)] or in healthy dogs [171 (91-380)]. The Pearson correlation between basal cortisol and cortisol following ACTH-stimulation was positive in control dogs (p’=0.434), and was progressively higher in survivors (p’=0.64) and in non-survivors (p’=0.784).

Delta-cortisol concentrations were significantly higher in non-survivors [158 (76-537)] than in survivors [115 (29-362)]. No significant difference was observed between non-survivors and control dogs [124 (78-360)] and between survivors and control dogs. Interestingly, non-survivors showed a positive correlation between basal cortisol and delta-cortisol. However this correlation remained of low significance (p’=0.453).

ALDOSTERONE (EXPRESSED IN PMOL/L AND PRESENTED AS MEAN (MIN-MAX)), (Table I, Figure 2)

Basal aldosterone was significantly higher in non-survivors [280 (14-2515)] than in control dogs [51 (5-1169)] and in survivors [66 (14-1014)].

Aldosterone concentrations following ACTH-stimulation were significantly higher in non-survivors [1163 (255-4111)] than in control dogs [249 (55-2122)] and were higher than in survivors [604 (91-5540)]. The correlation between basal aldosterone and aldosterone following ACTH-stimulation was positive in control dogs (p’=0.639) and in survivors (p’=0.605) and increased in non-survivors (p’=0.927).
Delta-aldosterone was higher in non-survivors [770 (241-1596)] than in control dogs [158 (45-1241)] and in survivors [452 (77-5302)]. However, the differences were not significant. The highest positive correlation between basal aldosterone and delta-aldosterone was observed in non-survivors (p' = 0.773).

**NA, K AND NA/K RATIOS (Table I)**

Sodium and potassium concentrations and Na/K ratios were not significantly different between control dogs, survivors, and non-survivors.

**SYSTOLIC ARTERIAL BLOOD PRESSURE (Table I)**

The results failed to show any significant difference between control dogs, survivors, and non-survivors.

**CORRELATION BETWEEN DIFFERENT PARAMETERS**

Non-survivors showed a positive correlation between basal cortisol and basal aldosterone (p' = 0.729). This correlation was not observed in the other groups.
Discussion

The present study was carried out to evaluate adrenal responsiveness in critically ill dogs.

The relevance of the absolute value of basal cortisol and cortisol following ACTH-stimulation as appropriate indicators of the response to illness is unknown in dogs. For these reasons, we chose to compare adrenal response between different healthy dogs and critically ill dogs, which were then subdivided into survivors and non-survivors.

Because we wanted an overview of adrenal response in critically ill dogs, we obtained a heterogeneous population of patients. The previous report in veterinary medicine included only 20 dogs presenting a large panel of different disease [26]. In order to overcome the dispersion of data, we tried to determine correlation coefficients (Pearson correlation) between basal cortisol or basal aldosterone and adrenal response.

The control dog population was more homogeneous. We preferred compare critically ill dogs and healthy dogs in similar stress and hospitalization conditions than to refer to reference values.

In this study, basal cortisol clearly discriminated between normal and critically dogs, with higher concentrations in the latter. Nevertheless, the lack of significant difference between basal cortisol values in survivors and in non-survivors leads to qualify the influence of the severity of illness on the basal cortisol secretion.

Noticeably, human patients with critical disease and high baseline cortisol concentrations could have a low cortisol response to the ACTH stimulation test [1,4,12,17]. Our results did not identify a lower adrenal response to ACTH stimulation in critically ill dogs. Moreover, the highest cortisol values following ACTH-stimulation were observed in non-survivors. Interestingly, this high cortisol value following ACTH-stimulation was not associated with significantly increased delta-cortisol values in this group, as compared to healthy dogs. Yet, the network of evidence assembled here indicated a difference in adrenal response between the two subgroups of critically ill patients. The higher cortisol values following ACTH-stimulation observed in non-survivors could reflect a higher adrenal sensitivity to ACTH, induced by a higher endogenous ACTH secretion due to a more important inflammatory stress [15,24,25]. In addition, correlations between basal cortisol values and adrenal response (cortisol following ACTH-stimulation and delta-cortisol) were enhanced in non-survivors indicating a greater activity of HPA axis associated to the severity of illness.

In humans and in animals, most forms of stress or severe illnesses are followed by an immediate increase in ACTH secretion, which is followed a few minutes later by an important rise in blood cortisol concentrations. During severe illness, circulating pro-inflammatory cytokines, including IL-6, tumor necrosis factor (TNF)-alpha and IL-1beta lead to an increased production of ACTH and subsequently to an increased cortisol secretion. In humans, numerous other factors such as the noradrenergic system, vasopressin, serotonin, angiotensin II stimulate CRH secretion and ACTH production. Severe illness also reduces cortisol binding globulin concentrations, leading to an increase in free and active cortisol blood concentrations [22].
The influence of aging could be questionable in our study, because survivors were younger than non-survivors. Age-related alterations in the HPA axis regulation have been reported. Strasser et al. [33], and Rothuizen et al. [29] showed higher basal cortisol concentrations in old dogs than in young ones. In our study, critical illness induced an increase in basal cortisol values in survivors as well as in non-survivors. The difference was not significant leading to hypothesize that the influence of critical illness may be higher than the influence of age on the adrenal secretion.

We considered that variations of aldosterone concentrations after ACTH could complete the overview of adrenal function. In humans, an unexplained drop of adrenal aldosterone production has been reported in stress or severe illness [10,20]. In our study, basal aldosterone concentrations were significantly higher in non-survivors than in survivors and in control dogs. This observation taken together with the elevation of aldosterone following ACTH-stimulation values did not support a low adrenal response in aldosterone. In this study, systolic arterial blood pressure, Na/K ratio, sodium and potassium concentrations were not different in critically ill dogs as compared with control dogs at evaluation time and thus, did not stand as a potential underlying cause of the basal aldosterone variations [8,16]. The lack of variations in electrolytes and arterial blood pressure observed in our patients was probably due to the superposition of the underlying disease and prior therapeutic interventions.

It is still possible to hypothesize that our critically ill patients trend to be hypovolemic without hypotension, and this status could stimulate the renin-angiotensine-aldosterone system and consequently produce an increase of basal aldosterone concentrations. Other reports mention the ability of a sustained elevation of endogen ACTH to maintain high aldosterone concentrations within 48 hours of admission [15]. In non-survivors, the suspected elevation of endogen ACTH could explain observed high basal aldosterone concentrations.

A closer correlation was observed between basal aldosterone and adrenal aldosterone response (aldosterone following ACTH-stimulation and delta-aldosterone) in non-survivors. A similar observation was already made concerning the correlation between basal cortisol values and adrenal cortisol response in this group. In addition, the correlation between basal aldosterone and cortisol variations was higher in this group. Altogether, these observations underlined a greater activation of HPA axis in non-survivors as compared to either the control dogs or survivors.

It has been showed, particularly in septic shock, that human patients with RAI are unable to have an appropriate response to stress. In human patients with sepsis, high circulating concentrations of inflammatory mediators may play a role in the development of RAI through the inhibition of the HPA axis. In our study, we did not show a depressed adrenal response in our critically ill patients. However, we were unable to evaluate the response was appropriate for the patient inflammatory status.

**Conclusion**

In this study, we observed an activation of adrenal responsiveness in critically ill dogs. Adrenal response appeared to be enhanced by the severity of disease. Even though we could not determine if adrenal response in our patients was appropriate to their status, we could not establish in which patients glucocorticoid supplementation could be beneficial. Moreover, we could not determine if basal and following-ACTH cortisol concentrations are relevant to discriminate critically ill dogs, which may be treated with glucocorticoid. Monitoring the HPA axis in critically ill patients is challenging and lacks endpoints. We propose, as a further investigation, to include a more homogenous group of critically ill population (hemorrhagic or septic shock models). Moreover, low-dose ACTH stimulation could be performed, providing additional axis of investigation of the adrenal function [11,17,22,25]. Finally, plasma ACTH, other adrenal cortex hormones such as dehydroepiandrosterone sulphate [5], CBG, or free cortisol could be measured to obtain a complete overview [4,27].

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**References**