Glucocorticoid Receptor Density and Binding Affinity in Horses with Systemic Inflammatory Response Syndrome

Abstract

There were three objectives of this study. The first was to determine if commercially available fluorochromes could be used to determine the glucocorticoid receptor (GR) density and binding affinity (BA) in equine peripheral blood mononuclear cells. The second was to determine if there was a correlation between elevated plasma cortisol and GR density or binding affinity in healthy adult horses. The third objective was to evaluate the HPA axis in adult horses presenting with systemic inflammatory response syndrome (SIRS), and to determine where any alterations in HPA axis function occur in these patients compared to healthy adults. For the first part of the study, peripheral venous blood was collected from 3 healthy research horses on 3 days. Peripheral blood mononuclear cells were isolated using Ficoll gradient centrifugation. Phycoerythrin (PE)-CD44 was then used to extracellularly label leukocytes, and then an intracellular GR antibody was used to determine a baseline measurement of GR density and fluorescein isothiocyanate (FITC)-dexamethasone was used to determine binding affinity via flow cytometric analysis. Comparison of control samples to those for CD44, GR density, and GR binding affinity showed a statistically significant difference for all samples (P<0.0001, P<0.0001, and P<0.0001 respectively). This showed that the CD44, GR antibody, and FITC-dexamethasone could successfully be used to analyze equine peripheral blood mononuclear cells for GR activity.

For the second part of the study, an ACTH stimulation test was performed on 8 healthy horses in order to induce an increase in endogenous cortisol production. Plasma cortisol levels, GR density, and GR binding affinity were measured at baseline, 4, 8, and 24 hours after treatment. Median basal cortisol concentration was 4.9, range 3.2-6.1 μg/dl. This initially increased following ACTH stimulation to 5.6, range 4.8-7.4 μg/dl, then showed a significant decrease by 8 hours post ACTH administration to 1.4, range 1.1-2.7 μg/dl (P=0.0221). No correlation was observed between plasma cortisol concentration in healthy horses and GR density or binding affinity (r=-0.145, P=0.428 and r=0.046, P=0.802, respectively).

For the third phase of the study, horses (N=10) with systemic inflammatory response syndrome (SIRS) were compared to healthy, age and sex matched controls (N=10) presenting for lameness evaluation or ophthalmologic examination. Blood was collected from SIRS cases and controls on presentation to the Equine Medical Center. A CBC, serum biochemistry, and serum ACTH and cortisol measurements were performed. GR density and binding affinity were also determined. Nonsurvivors had a significantly decreased GR binding affinity (P=0.008) and demonstrated a trend towards an increase in the ACTH:cortisol ratio. ROC analysis was performed for serum ACTH and cortisol concentrations, the ACTH:cortisol ratio, GR density and GR binding affinity, and triglycerides to determine cut-off values associated with nonsurvival. These were then used to analyze this population using Fischer's exact test to determine the odds ratio (OR) associated with nonsurvival for each variable. This revealed that a serum triglyceride concentration greater than 28.5 mg/dl was associated with nonsurvival (OR=117, 95% CI, 1.94-7060). The other variables were not found to be significantly associated with nonsurvival, although a Delta BA% of less than 35.79% was found to be closely associated with nonsurvival (OR=30.33, 95% CI, 0.96-960.5). Additionally, a significant negative correlation was detected between the plasma ACTH concentration and Delta BA% (r=-0.685, P=0.029) and the ACTH:cortisol ratio and the Delta BA% (r=-0.697, P=0.025).

This study showed that nonsurviving horses with SIRS had a significantly decreased GR binding affinity compared to survivors, and a tendency toward an increase in their ACTH:cortisol ratios. This confirms that HPA axis dysfunction occurs in adult horses with SIRS as tissue resistance to glucocorticoids, and potentially relative adrenal insufficiency as well. These results suggest that there are horses with SIRS that might benefit from "physiologic" doses of synthetic glucocorticoids to complement their relative adrenal insufficiency in addition to their poor tissue sensitivity. Further research should focus on methods to more rapidly determine which horses might benefit from treatment with glucocorticoids on presentation, as well as to more accurately determine prognosis for survival.
INTRODUCTION Glucocorticoid (GC) therapy is the main treatment for systemic lupus erythematosus (SLE). However, some patients are resistant to these agents. Abnormalities of glucocorticoid receptor (GR) seem to be related to steroid resistance. This study evaluated GRs in T lymphocytes and monocytes of SLE patients by flow cytometry (FCM) using a monoclonal antibody (mAb) and FITC-Dex probes. Thirty-five patients with SLE before treatment and 27 age- and sex-matched normal controls were studied. Disease activity scores were determined before and after treatment and used to divide the patients into steroid-resistant (SR) and steroid-sensitive (SS) groups. Glucocorticoid (GC) therapy is the main treatment for systemic lupus erythematosus (SLE). However, some patients are resistant to these agents. Abnormalities of glucocorticoid receptor (GR) seem to be related to steroid resistance.