

Cytokine response to furosemide application in six Thoroughbred horses

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Introduction

Furosemide is commonly administered in horses prior to racing to reduce the incidence of exercise induced pulmonary hemorrhage (EIPH)¹ during the race through lowering of systemic blood pressure². Studies have demonstrated that furosemide has an immunomodulatory effect in humans and laboratory animals³ and there is recent data that it alters the mRNA expression of certain cytokines in racehorses. Among these are TNF- α , IFN- γ , IL-4 and IL-10.⁴ In the current study, our objective was to investigate the immunomodulatory effect of furosemide in the horse. We hypothesized that cytokine concentrations would be altered after furosemide application.

Methods

A crossover study design with a 14-day washout period between trials was used. Serum samples from six healthy Thoroughbred horses were collected through a catheter prior to intravenous furosemide application (1.0 mg/kg) or an equivalent amount of saline solution (0.02 ml/kg), as well as 4 and 24 hours after treatment. Serum samples were stored at -80°C and analyzed with a Luminex assay for 23 different cytokines (Eotaxin, FGF-2, Fractalkine, G-CSF, GM-CSF, GRO, IFN γ , IL-1 α , IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17a, IL-18, IP-10, MCP-1, RANTES and TNF α). Horses were fed every 12h to standardize effects of feed intake. A mixed effects model was used for statistical analysis with a cutoff for significance of $p < 0.05$.

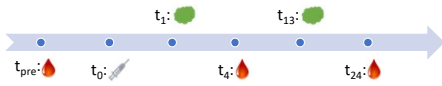


Figure 1: Chart of trial day procedure. Blood collection is indicated by (●). Treatment is indicated by (→) and feeding is indicated by (●).

Discussion

Based on the existing literature showing changes in mRNA expression in racehorses⁴, we hypothesized, that the concentration of these cytokines would also be altered by furosemide administration, which play a role in the inflammatory response. Our results don't support this hypothesis. The study prior had shown an increase of TNF- α and IFN- γ mRNA expression, as well as a decrease of IL-4 mRNA expression after furosemide application and racing compared to mRNA expression prior to racing and treatment. In that study IL-10 mRNA expressions increased in both groups⁴. Reasons for not seeing these changes in the current study, can be due to delayed or repressed mRNA translation, which can lead to altered cytokine concentrations beyond the 24 hour mark. Compared to the study prior, our study did not include an inflammatory event like exercise. Through an induced increase of inflammatory cytokine concentrations, a possible suppressive effect of furosemide administration on these concentrations would have been easier to detect. Also, one horse showed adverse effects to the treatment and approximately one third of the data from this study was below the lowest level of quantitation, which made statistical power weaker. A larger sample size would have also contributed to more statistical power.

Limitations

Analysis of mRNA expression for cytokines and their equivalent cytokine concentrations, ideally with a larger sample size, need to be performed simultaneously in future studies, to investigate when cytokine concentrations change or if mRNA is being degraded instead of translated. Furthermore, an inflammatory event should be added to the study design to better show potential suppression of certain inflammatory cytokines caused by furosemide application.

Results

Inflammatory cytokines

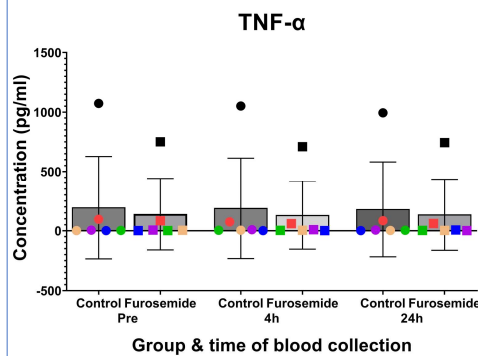


Figure 2: The graph shows TNF- α concentrations at 3 different times from a group treated with furosemide compared to a control group. Each color represents a specific horse. In the current study no significant concentration changes were seen for TNF- α ($p < 0.05$) within the first 24 hours after treatment.

IFN- γ

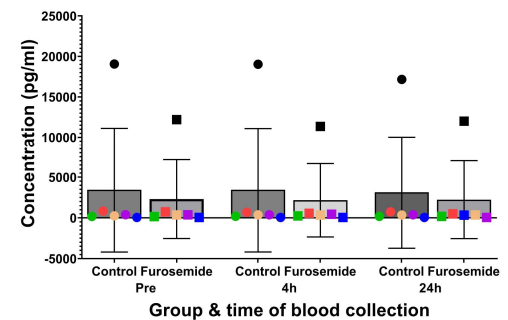


Figure 3: The graph shows IFN- γ concentrations at 3 different times from a group treated with furosemide compared to a control group. Each color represents a specific horse. In the current study no significant concentration changes were seen for IFN- γ ($p < 0.05$) within the first 24 hours after treatment.

Anti-inflammatory cytokines

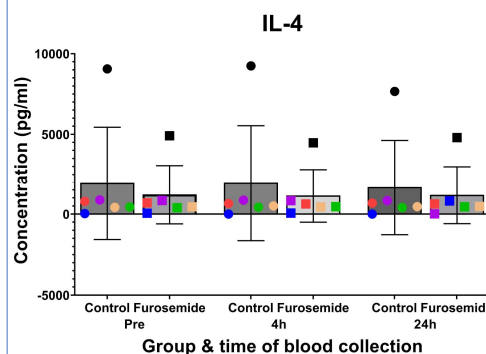


Figure 4: The graph shows IL-4 concentrations at 3 different times from a group treated with furosemide compared to a control group. Each color represents a specific horse. In the current study no significant concentration changes were seen for IL-4 ($p < 0.05$) within the first 24 hours after treatment.

IL-10

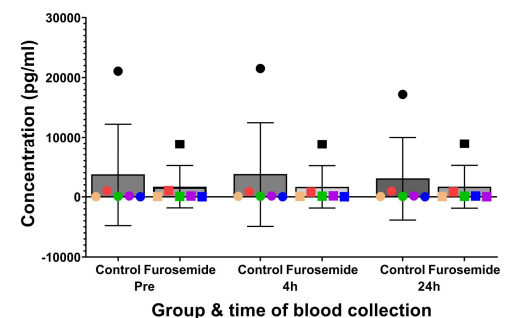


Figure 5: The graph shows IL-10 concentrations at 3 different times from a group treated with furosemide compared to a control group. Each color represents a specific horse. In the current study no significant concentration changes were seen for IL-10 ($p < 0.05$) within the first 24 hours after treatment.

No significant concentration changes were found in all 23 cytokines ($p < 0.05$). Significant effect of the horse in all 23 cytokines was found.

Acknowledgements

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