



Alpha-2 Macroglobulin for the Management of Equine Asthma- Summary Results of a Pilot Study

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Introduction

Equine asthma (EA) is a chronic respiratory disease of adult horses that results from exposure to airborne environmental allergens in susceptible horses. The role of infectious agents remains subject to debate.¹ The disease is characterized by an inflammatory response in the airways leading to mucous accumulation and bronchial constriction, leading to clinical signs that include coughing, nasal discharge, increased respiratory effort, and decreased performance/exercise intolerance.^{1,2} While a complete discussion of equine asthma is beyond the scope of this document, it should be remembered that equine asthma is a highly prevalent equine respiratory disease that can significantly impact both horse's quality of life and can be the cause of poor performance/exercise intolerance. Equine asthma is divided into two categories based on severity: mild-moderate equine asthma or inflammatory airway disease (IAD) and severe equine asthma or recurrent airway obstruction (RAO). Inflammatory airway disease can affect horses of any age and results in clinical signs less severe than recurrent airway obstruction, which most often affects horses over 7 years old.^{1,3,4} Equine asthma is a highly prevalent disease: IAD may affect 68-80% of horses based on bronchoalveolar lavage (BAL) and RAO may affect 14-17% of horses.^{5,6}

Pathogenesis and Diagnosis

The pathogenesis of EA has not been fully elucidated.¹ A wide range of non-infectious environmental agents may all contribute to the development and exacerbation of EA— molds, fungi, dust particles, noxious gases.⁷ Genetic predisposition may contribute to the development of EA, especially for RAO.⁸ A recent study demonstrated that obesity, a body condition score > 7, is a statistically significant risk factor for developing EA.⁹ Though a single factor cannot be identified that leads to EA, the multiple factors involved result in a disease characterized by dysregulation of inflammatory cell homeostasis in the airway of horses with EA.^{1,2}

Equine asthma can be diagnosed using a variety of different methods. A presumptive diagnosis can be made based on the typical clinical signs and response to appropriate treatment. Endoscopy can reveal the presence of excessive tracheal mucous. Airway cytology assessing fluid obtained when performing bronchoalveolar lavage (BAL) is often used to confirm a presumptive diagnosis of EA based on clinical signs and endoscopy findings. Horses with IAD will typically demonstrate a mild to moderate increase in neutrophils, eosinophil, and/or mast cells, while horses with RAO will show moderate to marked increases in the neutrophil count.¹⁰ Pulmonary function testing is not frequently performed on clinical cases of EA but has shown impaired lung function and gas exchange.¹¹

Treatment

The evidence in the literature for treatment of EA is limited considering the prevalence of the disease. The treatment of horses with EA focuses mainly on decreasing and controlling airway inflammation.¹ Management changes— including limiting exposure to dust and decreasing other airborne particles by increasing ventilation in barns— can be effective adjunct methods to limit airway inflammation. For many years it has been customary practice to treat horses with EA with corticosteroids. Both systemic and inhaled corticosteroids may be effective in mitigating the clinical signs associated with EA, especially RAO. A study looked at the effects of both a systemic and an inhaled steroid on the cytology of BAL fluid and the clinical signs of IAD in a group of horses. In this uncontrolled study with a limited number of horses, the corticosteroids did not statistically significantly change the cytology of BAL fluid, improve the clinical signs of IAD, or have a lasting effect 3 weeks after treatment was discontinued.¹² More recently studies with a different inhaled steroid, ciclesonide, have demonstrated improved clinical signs in a group of horses with mild to severe EA, and that drug has received approval from the Food and Drug Administration- Center for Veterinary Medicine (FDA- CVM) for management of the clinical signs associated with severe equine asthma in horses.^{13,14}

Additional treatments for EA include the use of mast cell stabilizers like sodium cromoglycate and interferon alpha to decrease airway inflammation and improve the clinical signs of EA.¹ Bronchodilators, including systemic clenbuterol or inhaled albuterol or ipratropium bromide, to reduce coughing and assist with mucociliary clearance of mucous can be beneficial, especially when combined with corticosteroids.¹ A study looked at the effects of supplementing the diet with omega-3 fatty acids and switching to a low dust feed compared to the low dust feed alone and demonstrated a more rapid improvement in clinical signs of both IAD and RAO when compared to the low dust diet alone.¹⁵

Initial Safety Study

Based on the mechanism of action of alpha-2 macroglobulin ($\alpha 2M$) resulting in acting as a naturally occurring, non-corticosteroid anti-inflammatory molecule, the decision was made to examine the safety and clinical effects of autologous $\alpha 2M$ on horses with EA.¹⁶

A single arm, open label study on eight horses diagnosed with mild EA based on clinical signs and BAL had blood drawn and processed according to the instructions with the Alpha2EQ system from Astaria Global. Each horse was evaluated and assigned a baseline weighted clinical score (WCS) based on nine respiratory related clinical signs, modified from a published study, prior to initiating the nebulizations.¹⁷ The nebulization protocol consisted of a series of six (6) nebulizations using 3 mL of Alpha2EQ product, diluted to 6 mL with sterile saline or lactated Ringer's solution (LRS), administered at 48-hour intervals. On Day 7 following the last nebulization a final WCS was obtained by the investigator. Each horse had temperature, pulse, and respiratory rate (TPR) assessed prior to and one-hour post-nebulization and was observed for any adverse events, including, but not limited to, increased coughing or epistaxis. No additional medications were added, or management changes made during the study.

None of the horses had any adverse events. The WCS decreased from baseline in 7 of the 8 horses. Based on the positive results from this study a larger study was performed.

Pilot Study

This study used the same nebulization protocol and objective outcome measures as the initial safety study in a larger group of horses with a diagnosis of either IAD or RAO based on clinical signs and/or BAL. It was a single arm, open label, non-controlled study with 54 horses from 8 different sites enrolled. Each horse had to have demonstrated at least one clinical sign of EA for 14 days or more prior to enrolling, have a history of at least two previous episodes of labored breathing at rest, and a history of demonstrating improvement in clinical signs with an appropriate treatment for EA. All horses had to have an initial (baseline) WCS > 7 prior to initiating the nebulization protocol. If they were on medications for EA yet still had a WCS > 7 they were allowed to remain on those medications but with no increased dosages. No additional medications or management changes were permitted from the time of enrollment until the Day 7 post-nebulization WCS assessment. A successful outcome was defined as a decrease in WCS of $\geq 30\%$ from baseline and a final WCS ≤ 7 .

Of the 54 horses enrolled, 34 met all the study criteria and had all the data for analysis. (Fifteen horses did not have the required WCS assessments and 5 horses experienced a management change during the study period.) One of 54 horses experienced a transient episode of increased coughing associated with one nebulization. None of the horses demonstrated a clinically significant change in TPR following nebulization, and no horse demonstrated epistaxis. Thirty of the 34 horses (30/34, 88.2%) met the definition of success. All 34 horses had a Day 7 post-protocol WCS lower than the baseline score, and 32/34 (94.1%) horses had a decrease in WCS of $\geq 30\%$.

The study was designed to assess the response of the horses over an approximate three-week period. However, many of the horses have demonstrated a decrease in the clinical signs of EA for between one and six months, off all other medications, following the initial series of nebulizations. Several of the horses, principally those with higher initial WCS, that begin to show a return of clinical signs of EA have been maintained with a single nebulization once every four (4) weeks. A small number of horses have received intermittent doses of medications to treat specific clinical signs associated with EA while being maintained on $\alpha 2M$.

Summary

There are limitations to this study: it is an open label, non-controlled study in a limited number of horses with clinical equine asthma. However, as a proof-of-concept study assessing the safety and clinical effects of autologous $\alpha 2M$, no significant adverse events were reported, and substantial clinical benefits were demonstrated in mitigating the clinical signs of EA. Using a weighted clinical score, autologous $\alpha 2M$ demonstrated a success rate of 88.2% (30/34). This product may offer a new option for managing the clinical signs of EA as a non-corticosteroid anti-inflammatory alternative for use in affected horses.

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